

Targets for future arms control

Before East-West talks on arms control resume, there is a strong case for an examination of what the two sides should be aiming at — even for an agreement on the subject.

PREDICTABLY, not much has been heard of arms control in the past few weeks. For all the importance rightly attaching to the question how best to reach an accommodation between the Soviet Union and the United States on the range of issues left suspended at Geneva a year ago, it was never likely that the US electorate would let the presidential election be determined by the answers of the different candidates. And there is a sense in which the indifference is well-placed. Even if a re-elected President Reagan should be discovered to be more flexible than he has sometimes seemed to be, as on issues such as negotiation from strength, everybody knows that it takes two to make an equitable agreement — and nobody knows what the putative partners in negotiation are now thinking. But a new president, whatever his virtues, would be no more able to conjure agreement out of thin air, and would in any case have to spend time learning what kind of agreement would be acceptable. So what can people, in the United States and elsewhere, hope to be doing while waiting for the dust to settle?

The simple but important task that needs to be taken up is that of defining a stable and possibly attainable goal for arms controllers in the medium term, say by the end of the century. Such an exercise is not as academic as it seems, for there is a sense in which proposals for new arms control agreements between the Occidental super-powers or within larger groupings, reasonable enough in themselves, turn out to be insubstantial when set against the pattern of changing military technology and the complexity of the world in which international conflicts of interest must be supposed to continue to exist. This is the spirit in which the notion of a comprehensive ban on nuclear testing, desirable (and nearly attainable) in the early 1960s, would no longer be safe (on its own), let alone feasible. Similarly, because the Salt agreements (both I and II, the latter and the more important never ratified) say nothing about missiles of intermediate range, strategists have been stuffing Europe with SS-20, Pershing and cruise missiles.

These disheartening developments are not as surprising as they appear. This side of that utopian state of affairs in which international conflicts of interest have been subsumed in universal sweetness and light, no single arms control agreement of the kinds now talked about at negotiations can on its own be stable. A comprehensive test-ban treaty would survive only until one of the participants considered its position had been undermined by the development of other military technologies than those for bomb construction. The Non-Proliferation Treaty, which mercifully still holds, would not have much value if it were not backed up by a whole array of political restraints on potential bomb-makers, but would not last long if more than one or two of its signatories defected. Nuclear-free zones in Europe would probably survive for as long as the countries directly concerned agreed not to change their external relationships significantly. And even the design of a more general package of arms control measures to limit the damage that the superpowers may inflict on themselves (and others) requires, for stability, a clear understanding of what political changes will be allowed without bringing the whole agreement tumbling down.

Put directly, the problem is this. Is there any arms control regime that can be expected in Europe to survive, say, the transfer of one Central European state from one alliance to its opponent?

As a corollary, is it possible to conceive of a regime for nuclear arms control in Europe that will allow conventional wars to proceed conventionally? And if not, what understandings about political change must accompany what the arms controllers agree? These important questions have been given far less attention than they deserve.

But do not the Helsinki agreements (on European security) serve just such a purpose? They formally require of signatory governments such liberality in regulation of people's freedom that, if the agreements were enforced, many potential international conflicts would never surface. The trouble, as the world knows, is that the Helsinki agreements are a dead letter, especially in Eastern Europe. But an attempt to breathe new life into them, perhaps by new administrations in the United States and the Soviet Union, would not be a waste of time.

The safest assumption (which some would think wild) is that Helsinki could yield, by 2000, an understanding that moderately paced political change would be permitted among the lesser allies of major powers, perhaps against a background of steady economic improvement (also one of the Helsinki objectives). Whatever the permissible pace of change agreed, it is unthinkable that either side would agree to the entire abandonment of nuclear weapons within Europe, while even some European states (France, for example) would think twice on that subject. The best realistic bet is that great-power deterrence would persist, but preferably based on relatively invulnerable submarines, and that nuclear warheads actually based in Europe would be limited to, say, a score on each side. Inspection and verification could be by means of human inspectors (one per warhead) and by tight control of military nuclear explosives. Such a force of warheads would have the limited purpose of helping defence against overwhelming conventional attack, and of alerting allies.

In such a setting, chemical weapons (more attractive to the generals as weapons in conventional wars) could and should be outlawed, while the regulation of nuclear facilities within Europe could obviously be enormously simplified. For the inter-continental powers, the accompaniment of this regime would plainly be such a tight restriction of strategic warheads — say 100 — that only bases in invulnerable submarines would make sense. In such a regime, it would obviously be sensible to aim at a cut-off of military production — no skin off the noses of the major powers but a help against proliferation. There are two views of this prospect — one that it is a cowardly over-modest goal at which to aim, the other that it is idealistically ambitious. The second opinion, unfortunately, is nearer the truth. □

New academic tests

British universities have to share funds for research. Self-choice is best.

BRITISH universities seem to have embarked on their new academic year in a mood blended of resignation and combativeness. After several years of shrinking budgets, and after a summer filled with threats that there is worse to come, even the hope of a period of stagnation, called "level funding" in the trade, seems almost too much to ask for. But, since the appearance of the University Grants Committee strategy



document a few weeks ago (*Nature* 20 September, p. 193) some university administrators and their academic colleagues have indulged the fancy that the government is about to relent. What has happened is simply that the grants committee has taken a more robust view than in recent years of the universities' problems, and declared itself sympathetic to their needs. Specifically, it has made it plain that it does not passively accept that the universities can bend ever more pliantly to the government's demands (to train more engineers, for example) on a fixed budget. But as the experience of recent years has shown, even the strongest case may not prevail against a government strapped for cash. Lord Flowers, chairman of the Committee of Vice-Chancellors and Principals, was right to remind his constituents a week ago that this is not a "battle won, but a battle joined".

Euphoria is contagious, but dangerous. The debate arranged for 25 October in the House of Commons, on which many hopes are pinned, is almost certain to be a disappointment for the universities. Having lost their political friends in the 1970s, they are unlikely to be much comforted if the government's political opponents seize on what has been done to them as yet another stick with which to beat the administration over the head; or if, as seems likely, the government disarms criticism of its handling of British higher education in the past five years by a show of generosity, perhaps by promising an increase of the budget for university equipment. The test that those in higher education should apply to what is said in the House of Commons is that of whether the debate reveals an understanding that higher education (not just in universities) is a national resource and an economic and social necessity, not just a way of insulating a bunch of academic layabouts from the rigours of the labour market. (Tenure irrationally remains everybody's *bête noire*.)

Meanwhile, the universities have work of their own to do. The grants committee's document is rhetorically encouraging but full of pitfalls for the unwary or the indolent. Thus nothing substantial is said about the chief iniquity of the past three years — the imposition of student quotas that individual universities might exceed only at the risk of being penalized financially. The inference is that the grants committee will not in future enforce the rules its predecessor committee promulgated, which is a welcome development. The result should be that many universities will be able to make more economical use of the resources at their disposal by allowing departments starved of students to operate at full capacity. The snag, also only implicit in what the grants committee says, is that individual institutions will find themselves engaged in a complicated attempt to second-guess the grants committee. Taking in more students may help financially, if only at the margin, but may hazard an institution's capacity to compete for research support in future, but under a set of rules that has yet to be defined.

The issue is complicated and potentially contentious. The grants committee acknowledges, perhaps even overestimates, the importance of its financial support to the conduct of academic research in Britain. And now, when it is generally agreed that the dual mechanism of research support (from the committee and, in parallel, by means of project grants from research councils) has broken down, the committee says that it will in future be more discriminating between universities. All that makes sense, if only because, on paper, the present system provides all institutions with the general support that should enable them to compete for project grants in circumstances in which it is also generally understood that some are more able to compete successfully than others. Indeed, the grants committee has, over the years, implicitly trimmed its sails to this wind by being more generous to some universities than others while declining to say why, no doubt in the knowledge of the ructions there would be if universities had grounds for thinking that research support was being "earmarked", a synonym for the act of telling universities what to do and thus a gross intrusion into academic freedom.

So by what criteria will the grants committee discriminate among British universities in the provision of research support? By all accounts, the question has not been settled. All that the

committee has said in public is that it will devise criteria that are distinct from those followed by the research councils in their provision of (mostly) project grants. Obviously that makes sense, for otherwise it would be just as well to replace the research councils as a whole by the grants committee. But what other yardsticks than peer-review, and a general sense of "timeliness and promise", can there be?

Here is one interesting and subversive way in which the grants committee might choose to solve the problem it has set itself. Why not, to begin with, take universities' own estimation of the reasons why they deserve research support? At first, the result would be that universities would share the research component of the grants committee's budget in much the same proportions as at present, and so might be tempted to assume that nothing had changed. But in making a case for research support, the prudent among them would be compelled to explain why their research proposals should be taken seriously. And the consequences of that could be enlivening. For it would turn out that universities that were capable internally of discriminating between good and less good research would be more likely to succeed in the second round of the proposed competition. And those which had the flair to spot new lines of promising research at an early stage, and the willingness to commit internal resources to their support, would be found to emerge with extra kudos from such competitions. So, properly handled, the grants committee's ambition to devise alternative criteria for assessing research support may turn out to provide two of the benefits conspicuous by their absence in recent years from the British system — internal mechanisms for deciding what backing should be given to what research, and opportunities by which institutions can improve their standing by their own wit. That such a system would also lend substance to the notion that universities are autonomous institutions even in the uncomfortable sense of that word is not the least of its attractions. □

Irish disunity

*Bombs apart, Anglo-Irish talks are hopeful.
Education deserves attention.*

THE unsuccessful attempt to assassinate the British cabinet last week will have one important and beneficent consequence — that of further stimulating the search for an accommodation between the governments of Ireland and the United Kingdom over the Ulster problem. By all accounts, the weeks preceding the bombing of the Brighton hotel in which four people were killed were cheerful times for the officials labouring on the details of an agreement. What seems to have happened, on the British side, is that one minister, Mr James Prior, has been replaced (at his own request) by another, Mr Douglas Hurd. In the nature of this, the most disheartening job in British politics, new policies require new men, who usually have only one chance at a solution. Mr Prior's chief initiative, the consultation between Irish politicians called a forum, was less successful than it might have been because of the absence of the Ulster nationalists and the intransigence of the Irish nationalists. Within the same framework, Mr Hurd appears to be putting his energy into the building of common institutions, the touchstone of Irish hopes two years ago.

While it is remarkable (but unremarked) that many Irish institutions (rugby football and the administration of lighthouses, for example) have survived Irish partition, it tends to be forgotten that Irish higher education has preserved many of the appearances of commonality. As European citizens, Irish students can enter British universities without paying the penal fees exacted from other foreigners, but the Irish Government will not help with maintenance costs. Roman Catholic students from Ulster tend to seek higher education south of the border, when they are welcomed as if Irish. There is the basis for a deal between the two governments on this trade in young people's aspirations, as well as for an understanding that, for the purposes of research, the two countries should count as one. That should be at least one part of the package of proposals Mr Hurd is hoping to have assembled by next month. □

Fast reactors

Japanese plans delayed despite Clinch River bonus

Tokyo

JAPAN'S fast breeder reactor (FBR) research programme is running into financial difficulties, despite the recent gift of Y200-million-worth of unwanted research equipment and instruments from the US Government's abandoned Clinch River project.

The difficulties are caused by a massive increase in estimates of the construction costs for Monju, a 300-MW sodium-cooled prototype FBR to be constructed near Tsuruga in Fukui Prefecture. Monju is the key project in the FBR development programme and a major step forward from the 100-MW experimental reactor Joyu, which went critical in 1977.

Ground clearance operations have begun at the Monju site after several years of geological and seismic surveys. But construction costs have now risen from the 1980 estimate of Y400,000 million (US\$1,600 million) to around Y590,000 million (\$2,500 million). Responsibility for the project rests with the Power Reactor and Nuclear Fuel Development Corporation, a large research organization attached to the Science and Technology Agency. Funds for the project are not, however, entirely provided by the government, around 20 per cent coming, under a 1980 agreement, from privately-owned electricity utilities, largely through a power source development tax.

Now, the Science and Technology Agency wants to pass on a third of the extra costs to the utilities. They, however, have refused point blank to increase their contribution, arguing that any further funds could be found only by increasing electricity charges — something the government is anxious to avoid.

The shortage of funds is likely to hit the project hard next year when construction should move into top gear so as to finish by 1990. At present, given that the Japanese Government is already running an enormous budget deficit, the most likely option is to postpone the completion date by up to 5 years and to slow down the rate of construction to match availability of funds.

The pessimism over completing Monju on time comes just as the federation of electric power companies and the big five nuclear construction companies have launched a new Y6,000 million (\$25 million) study project to choose the basic design for Monju's successor, a 1,000-MW demonstration reactor expected to be ready by the end of the century. The three-year study will aim to make the choice between a loop type design, in which heat from the primary sodium coolant is transferred to a secondary liquid sodium system outside the reac-

tor, and a tank type in which the heat exchanger is located inside the reactor. The two reactor types are being researched by groups led by Mitsubishi and Hitachi-Toshiba respectively.

The key to success is to find some way to cut the enormous construction costs of the FBR, currently estimated at around two and a half times that of a conventional light water reactor. One way in which this will be

Danish physics

Niels Bohr on a shoestring

Copenhagen

THE Niels Bohr Institute for nuclear and particle physics in Copenhagen, which celebrates the centenary of Bohr's birth next year, is in dire straits because of opposition to nuclear power in Denmark. That, at least is the opinion of one of its professors, nuclear theorist Aage Winther. For the result is poor student enrolment in physics at the University of Copenhagen (of which the Niels Bohr Institute is a part), and some departments with five physics lecturers per student. Because university research funds are distributed in rough proportion to student numbers, these are hard times for the Bohr Institute.

There have been no new research posts at the institute for seven years. With a staff of 50, the institute needs one or two posts a year to keep alive, Winther reckons. A clutch of retirements begins in five years' time, but meanwhile, the institute needs perhaps 10-15 new staff. Will it happen? Winther throws up his hands in a gesture of doubt. Sad news for a city that gave its name to the statistical interpretation of quantum mechanics and for the institute that housed Europe's first cyclotron. The Niels Bohr also began the European Organization for Nuclear Research (CERN), now based near Geneva, as a theoretical group in 1951.

The trouble lies not only with the opposition of the young to things nuclear and the fashion (as Winther describes it) for the new biology. The Danish university system, in which the Niels Bohr is enmeshed, is in something of a mess. First degrees typically take 8-10 years to complete, with some students stretching out their time to the age of 34. After such a long first degree, there are very few post-graduate students.

Also, as in many countries, research funds have been pared down "to the level of counting pencils and erasers". The government expected the universities to perform major surgery on themselves, says Winther. "But how can we do that with our

tackled is through a joint US-Japanese project, agreed in principle this week, to perfect methods of driving the sodium coolant by electromagnetic forces rather than by a conventional pump.

Technical specifications for the reactor are to be examined by the French company Novatome, for although enthusiasm for FBRs remains great in Japan, the technology is still a long way behind that of France. The French began operation of a 250-MW prototype FBR, the Phénix, in 1972 (at the same time as a similar reactor at Dounreay in the United Kingdom) and expect to have Super-Phénix — the world's first demonstration FBR — ready by 1985, some 15 years ahead of any Japanese competitor.

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democratic structures?" All university committees have 25 per cent representation from students and 25 per cent from technicians, says Professor Teder Olesen Larsen, chairman of the national science policy advisory committee. "With such a broad structure the only decisions the universities can take are decisions to do nothing."

Thus the Niels Bohr Institute and many other important laboratories in Denmark's universities, have been submerged — except where they can fight for special support. This is often distributed by the government in "cigar boxes", a Danish phrase for special-purpose funds administered separately from the 10 per cent or so of research money that comes through the Danish research council system. Thus when essential moneys were allocated for the re-instrumentation of Danish laboratories, the money came from a "cigar box", where research council priorities were not easy to take into account. With so many special funds, the Danish research support system has now become "Kafkaesque", says Olesen Larsen.

The Niels Bohr is hoping to insulate itself from these problems in two ways. First it has negotiated a deal with the government that will give it more funds than its low student intake would normally entail. But the money will go in a lump to the university administration, and it is yet to be seen whether it comes the institute's way, says a cynical Winther. But as a second string, the Niels Bohr is hoping to raise more external support — which, with 50 mostly foreign visiting scientists, is already large. "We hope to make a success of our 100th anniversary celebration next year", says Winther, "by appealing to our external benefactors". Among them have been the US Ford Foundation and National Science Foundation; the Nishima Foundation of Japan; and the Italian national institute for nuclear physics (INFN). They should expect a few discreet Danish knocks on their doors next year.

Robert Walgate

European biotechnology

Community seeks new funds

Copenhagen

THE Governments of Austria, Switzerland and Sweden have asked the European Commission if they may join in the European Economic Community's next biotechnology programme, even though none of them belongs. The decision rests with the European Council of Ministers but the signs are that the applicants will be refused.

If so, this would be an inauspicious start to the 154-million-ECU (£130 million) five-year programme, which has as yet not an ECU (European currency unit) to call its own. The research plan, called the "Research Action Programme" (RAP) in biotechnology, has been conceived as a development and sixfold amplification of an existing Community biotechnology programme devoted to agriculture and food, the "Biomolecular Engineering Programme" (BEP). BEP has had "a tremendous impact" on developing collaboration among European research groups in plant sciences, said one senior participant speaking at a meeting last week to take stock of the programme.

Others, however, claim that while BEP has certainly been good for Europe, its benefits — new collaborations — will fade away when BEP ends (in March 1986) unless new funds are found to pay the costs of travel, meetings, training and materials now supported under BEP. Participants point to an earlier successful programme on photosynthesis, which collapsed when the programme finished.

One of the successes claimed for BEP last week is that work on the mitochondrial DNA of plants is now more advanced in Europe than elsewhere, partly as a result of the collaborations that have been supported. Without claiming sole credit, officials were gratified last week to hear from Professor Jeff Schell and his colleague Professor Marc van Montagu that *Agrobacterium* plasmids can be cloned into a monocotyledonous plant, *Asparagus officinalis*.

It seems to be agreed that BEP is responsible for the stimulation of *Agrobacterium* research in Europe and for establishing joint research programmes between groups that would otherwise have been working independently and in duplication. "The money has been better spent than it would have been in separate national programmes", said one participant.

BEP and, by extension, its successor, is not, however, without critics. Professor Schell, of Cologne and Ghent, doyen of the *Agrobacterium* vector for introducing foreign genes into plants, considered that the funds available (15 million ECU from Brussels) have been too thinly spread, but fears that if there were a massive increase in the budget, the money might be wasted among the undeserving masses.

Commission officials say, however, that

the objectives of the new programme are different, and that in the plant sciences, the best groups and programmes have already been identified. RAP would develop the programme, but also support initiatives in bioreactor technology (in particular for multi-enzyme, multi-phase and co-factor-requiring reactions), vaccines, hormones, gene transfer in animals, microbiology, the methodology of animal cell cultures (for monoclonal antibodies), new methods for

Danish collaboration

Europe seems long way south

Copenhagen

THE Danish minister for education and science, Mr Bertel Haarder, is to make a strong protest at the next European council of research ministers at the way in which France and Germany appear to have decided between themselves where to put the proposed European Synchrotron Radiation Source (see *Nature* 4 October, p.399), trading a trans-sonic wind tunnel for Cologne against the ESRS for Strasbourg or Grenoble.

Denmark had offered to site ESRS at the Risø atomic research centre, and had offered Dkr 350 million (£25 million) to help pay for it. But now Denmark feels its proposal was not properly assessed.

"We are very very upset", said Professor Teder Olesen Larsen, chairman of the Danish advisory council for science policy, last week. As well as Haarder's protest in Brussels, Denmark is to raise the issue at the annual general meeting of the European Science Foundation (ESF) in Strasbourg in November.

The ESRS project has been managed by ESF since 1979, and site proposals should have been assessed and compared by an ESF technical committee. In Denmark, it is now felt that this scientific assessment never took place, or at least was not taken seriously. A detailed Risø proposal has still not received a reply said Olesen Larsen.

The Risø proposal is generally believed in Denmark to have been technically excellent, and to have had a good chance of success if purely scientific criteria had been involved. "We would be very disappointed if a decision was reached by Franco-German agreement", said Olesen Larsen. "We are not going to be dictated to in that way." The French research minister, Hubert Curien "has a responsibility" at the very least to put the Franco-German proposal before the ESRS technical committee. If nothing is done to heal wounds there could be "very strong and nearly disastrous consequences for ESF", Olesen Larsen said.

According to Professor Alan Mackintosh, an ex-director of Risø now at the University of Copenhagen, Denmark

toxicological testing and other fields of "basic, pre-competitive" research.

Whether funds for the new programme will materialize is, however, still an open question. France, West Germany and the United Kingdom are objecting to the cost, even though all governments gave outline approval last April. Now the European Commission is trying to persuade ministers to open their purses by quoting the poor opinion of European biotechnology expressed by the US congressional Office of Technology Assessment, published in January this year (see *Nature* 307, 402; 1984).

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has a good record in synchrotron radiation research. Indeed, the latest design of ESRS was coordinated by a Dane, Professor Bronislav Buras, at the European Organization for Nuclear Research (CERN) near Geneva. The Nordic countries are also dismayed that they have never had a major European research facility (except the ionospheric laboratory, EISCAT). The complainants are, however, more reticent about the urgent domestic case for Risø as a site. It is one of those big laboratories originally devoted to nuclear research that needs to find new goals. ESRS would have suited it very nicely.

Risø's problems have been sharpened by the tacit Danish Government decision to abandon all hope of developing nuclear power stations in the light of the recent vote by the Social Democratic Party (SDP) (now opposing the centre-right coalition government) against nuclear power. Taking SDP together with the centre ("Radical") party, there is probably now a majority in the Danish Parliament against all nuclear development. For the first time in years, the Danish Prime Minister failed to refer to a nuclear future for Denmark in the opening speech to Parliament this month.

Another question-mark to place against Danish interest in ESRS could be the Danish commitment to another European institution, the European Molecular Biology Laboratory (EMBL) at Heidelberg. Denmark has kept its continued membership of EMBL in doubt now for three years — and that doubt will continue if Olesen Larsen's science policy committee has its way. A committee of biologists had recommended in August that Denmark should finally announce its long-term confidence in and commitment to EMBL, which costs the country Dkr 3 million (£210,000) a year; but the committee has rejected that advice, and instead advised Minister Haarder to leave the matter open for another three years — and meanwhile encourage more Danish molecular biologists to use the laboratory. Haarder has yet to decide which advice to take.

Robert Walgate

Japanese conservation

Aphrodisiacs and whales excepted

Tokyo

PRINCE Philip, Duke of Edinburgh, visiting Tokyo in his capacity as president of the World Wildlife Fund, has called upon Japan to take more vigorous action to abide by the Washington Convention — which regulates international trade in endangered species. His comments add to savage criticism of Japan's disregard of threatened species voiced at last week's Asian Pacific regional meeting of the convention — and comes when the Japanese Government is in a quandary over what to do about the cuts in whaling quotas agreed earlier this year by the International Whaling Commission (IWC).

The regional meeting of the convention on international trade in endangered species, held in Kuala Lumpur, ended with the adoption of a resolution severely criticizing Japan for failing to enforce the convention, which permits trading in endangered species only when official permission has been given by both exporting and importing countries. Japan is one of the signatories but has never actually enforced the regulations properly.

Critics at Kuala Lumpur pointed out that, despite the great efficiency with which Japan controls the import of manufactured goods, no experts are provided at customs offices who can check the status of a species or the validity of documents showing where animals have come from. The result is that Japanese traders have found it easy to deal in a range of endangered species — in a recent case, it was found that extremely rare lion tamarins (*Leontopithecus rosalia*) had been brought from Brazil with forged permits — as well as being able to sell large quantities of ivory, furs and tropical fish.

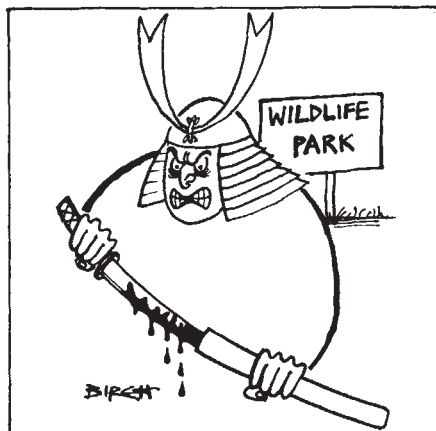
On top of lax administration of existing regulations, the Japanese Government has excluded from control 14 species recognized as endangered by the convention on the grounds that they are "vital for domestic industry". Chief among the excluded species is the musk deer, which has now been exterminated in most of India and is found in only a few isolated areas of the Himalayan states of Nepal, Sikkim and Bhutan.

Despite the real risk of extinction, the enormous prices that Japanese dealers will pay for the male deers' musk gland meant that around 3,500 adult male deer were killed last year. Probably four times that number of deer died when the young animals and females that fall into poachers' traps are included. All this to supply the ingredients for a traditional Chinese medicine of very doubtful efficacy.

The Kuala Lumpur meeting's resolution is not on its own expected to have much influence; it had been expected and discounted in advance by the Japanese. A representative was sent only to the first day of

the two-week meeting. Later, financial restrictions were claimed to have prevented fuller attendance. Of the other 16 Asian-Pacific member countries, only Iran was absent.

Prince Philip, on his visit to Japan, also said he tended to the pessimistic view that whale stocks are declining rather than the



optimistic view, taken by the Japanese, that commercial whaling can be continued without pause, and he argued that continued Japanese hunting of sperm whales would run "counter to the world consensus of opinion".

But the Japanese Government has already decided to permit sperm whale hunting in Japan's coastal waters on the grounds that it is a domestic matter. And, last week, the decision was made "in principle" to join the Soviet Union and Brazil in objecting to the cut in the minke whale quota agreed at the IWC meeting earlier this year (see *Nature* 310, 440; 1984). The objection will not, however, actually be lodged, and Japan will not actually break the quota agreement, until all attempts have been made to persuade the US Government not to retaliate by cutting fish quotas in US waters, as the United States has said it will do to any country disregarding IWC agreements.

A decision is unlikely to be reached until after the US presidential election in November. Right now, anyway, fisheries agreements are not a popular topic in Japan. Not only are talks with the Soviet Union over salmon fisheries stalled, but Japan has just been accused of systematic violation of US fishery quotas in the North Pacific region. The Alaskan office of the US Department of Commerce's National Oceanic and Atmospheric Administration has claimed that the Japanese fishing fleet has been deliberately misleading US observers placed on-board fishing boats and has been using secret telegraph codes to broadcast the presence of patrol boats.

It is expected that Prince Philip will raise conservation issues when he meets Prime Minister Yasuhiro Nakasone later this week.

Alun Anderson

US patents

Dying Congress aids innovation

Washington

THE 98th United States Congress ground to an inglorious halt last Friday after a frantic series of eleventh-hour compromises between the House of Representatives and the Senate that still left several major items of legislation unfinished and hence abandoned. Drafters worked around the clock, and such was the confusion that military jets had to be used to ferry back to Washington senators who, keen to start campaigning in their home states, had left the capital before a final vital vote authorizing next year's budget deficit.

Among last week's casualties were the Export Administration Act, which might have established a new framework for the control of high-technology exports but which resisted last-ditch efforts to reconcile House and Senate views, and the controversial Simpson-Mazzoli immigration bill.

But there were also successes. Manufacturers of "generic" pharmaceuticals won a last-minute battle in the Senate to strike out a section of the 1984 Patent Law Amendments Act that would have extended the scope of US process patents to cover products made outside the United States. At present, there is no straightforward way in US law (in contrast with the position in Europe) to prevent a "copycat" manufacturer from using a home-patented process abroad and then importing the end product for sale. As first drafted, the bill would have put a stop to that, but the generic manufacturers argued successfully that they would be prevented from conducting safety tests for their products if foreign suppliers were unwilling to testify before US courts about their manufacturing processes.

The bill as approved does, however, include measures to help inventors working in companies or universities. A new device, the Statutory Invention Registration (SIR), will enable an inventor to stake his claim for patent rights at little cost and without a full patent examination. An SIR would become "prior art", thus preventing a patent claim by a later inventor, and could be upgraded to a full patent later.

Another provision in the bill would alter the definition of prior art to exclude private communications between members of a research team, thus making it easier for team members to claim patent rights. Inventors will not have to work together to be able to claim joint inventorship, and different claims made by different inventors will all be entitled to the benefits of the earliest filing date. The bill would also establish a national commission on innovation and productivity to examine inventors' rights in relation to their employers.

Tim Beardsley

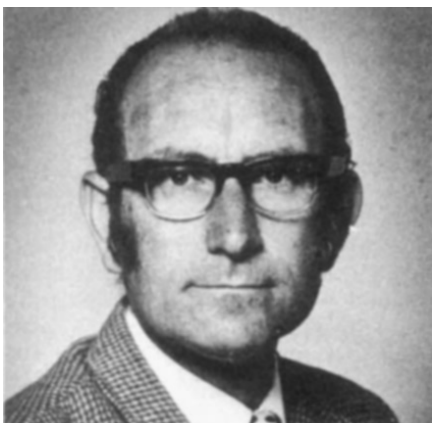
Soviet astronomy

Astrophysics in space

FRANCE is to build a 1-tonne gamma-ray telescope and imaging system to be launched by the Soviet Union aboard an Astron satellite in 1987. The project, which has been under discussion for some time, is covered by a special protocol signed earlier this month in Samarkand during the regular annual meeting of the Franco-Soviet working group on cooperation in space.

The announcement from Samarkand suggests that the name Astron, at present denoting the astrophysical satellite launched on 23 March 1983, is to become the name of a series. The first is basically a Venera-15 backup, stripped of its navigation gear and course-correction motor. The satellite carries an optical telescope (the largest to be orbited so far), an ultraviolet telescope, a spectrometer (produced by the Marseilles laboratory of space astronomy) working in the 1,100 to 3,500 Å range and an X-ray telescope-spectrometer in the 6 to 0.5 Å range. A high apogee (200,000 km) orbit is used to eliminate interference from the Earth's radiation belt and corona.

French participation in Astron-1 is confined to the X-ray study of normal stars and extended surfaces. The more spectacular results have, however, come from the Soviet part of the programme although — in the case of the anomalous behaviour of the X-1 Hercules X-ray signal — the observations were confirmed by the European Exosat satellite. X-1 Herculis is a double star consisting of the visible HZ-Herculis and a neutron star (the X-ray source). In the course of an observation session on 30 July 1983, the X-ray signal was found to have vanished, although visual observations from the Byurakan and Crimean observatories found no significant changes in the optical brightness curve. This suggested that the loss of signal was due to some change in the accretion disc of the neutron star, possibly in the angular distribution of the radiation.



DR César Milstein, one of this year's winners of the Nobel prize for physiology and medicine (see p.601).

During a subsequent observation session on 1 March 1984, the signal was found to be back to normal.

Other surprises have included the anomalous spectrum of 73 Draconis (in which the content of heavy elements (lead, tungsten and uranium) was considerably higher than theoretical predictions and the ultraviolet observations of the double stars SS-Auriga and SS-Andromeda, which indicated that the "hot" components have dimensions only a few per cent of the radius of the Sun. Also unexpected were the results of the occlusion of "fast burster" MXB 1728-34 by the Moon on 16 August, 1983, which suggested that, during the occlusion, the source had emitted constant X-radiation, not the expected sequence of

discrete pulses.

Astron is a major research project, involving a number of high-level Soviet institutes. It is therefore not surprising that these results have been given wide publicity in the general Soviet press, which claims every result as a major breakthrough. One newspaper even suggested that the work on 73 Draconis would lead to a greater understanding of the origin of metals.

It is also surprising that there are frequent errors in these popular reports. Thus the "hot" components of SS-Andromeda and SS-Auriga were said to have a temperature of 100,000K (not 60,000K), the lead and tungsten content of 73 Draconis have been described as "100,000 times higher than in the Solar System" and, on one occasion, TASS distinguished itself by locating the Astron telescope not in orbit but at the Byurakan observatory in Armenia.

Vera Rich

US health research

NIH further subdivided

Washington

CONGRESS last week approved a controversial research extension act for the National Institutes of Health (NIH) that creates three new institutes and for the first time sets out policy and general research directives for NIH component institutes. The bill was passed in the face of stiff opposition from NIH, which wanted to retain their existing freedom to set their own research priorities. The director of NIH, Dr James Wyngaarden, fears that the creation of new institutes will only add to bureaucracy and said that NIH will have to "find ways of accommodating" to the bill.

The principal change is the splitting of the National Institute for Arthritis, Diabetes, Digestive and Kidney Diseases into a new National Institute of Arthritis and Musculoskeletal and Skin Diseases and a separate National Institute for Diabetes, Digestion and Kidney Diseases. Part of the reason for the split appears to be the wish of private fund-raising organizations to have their favourite diseases incorporated into the name of an NIH institute. In addition, Congress has voted to create a new National Institute of Nursing: as politicians, congressmen are doubtless aware that few things are so likely to endear them to their electorates as the creation of a new medical institute.

Wyngaarden says the new institutes will create administrative costs without significantly benefiting the target areas: the work proposed for the new arthritis institute, costing \$80-100 million a year, is currently being carried out by the existing parent institute. The new act contains no special authorization for new bureaucratic costs. And the proposed research in nursing could best be done through existing mechanisms, according to Wyngaarden. But whatever the frustrations of a Congress that likes to involve itself in research administration, the ever-popular NIH have

little to complain about on budget; this year Congress has allocated \$5,000 million to NIH, a hefty increase on last year and way above the administration's request. But Congress does seem concerned about the threat of charlatanry. The legislation approved last week formalized for the first time the need for peer review of NIH research contracts and grants, and also requires administrators to set up mechanisms for investigating "substantial" scientific fraud.

Tim Beardsley

Health ethics

THE House and Senate also agreed last week to establish a Congressional Ethics Advisory Commission to study human genetic engineering and fetal research. The commission, to be modelled on Congress's Office of Technology Assessment, will report on scientific developments to Congress and the public, supported by a 14-strong biomedical ethics advisory committee.

The commission is seen as the US answer to the Warnock Committee in Britain, although it is expected to be more permanent.

Two studies are specifically mandated: whether the stringent federal protections for fetuses are appropriate for research, and the ethical implications of developments in genetic engineering technology that might be applied to humans.

As things stand, NIH are in practice barred from supporting research on *in vitro* fertilization and embryo transfer, and right-to-life ideologues in the administration who ally themselves to the Moral Majority are content to see things stay that way. The creation of the new commission is seen by its supporters as a vital first step towards new policy development.

Tim Beardsley

Acid rain

Parliament in power row

AN almost unprecedented row about acid rain has broken out between the Central Electricity Generating Board (CEGB), the monopoly generator of electricity in England and Wales, and the House of Commons Select Committee on Energy. The board's chairman, Sir Walter Marshall, last week complained that the select committee had "unfairly" pilloried his organization in a report on acid rain published a month ago (*Nature* 13 September, p. 94).

The House of Commons report differs from others on the subject including that earlier this year from the House of Lords, in the directness of its attack on CEGB, which is at one point accused of "ignorance or a deliberate attempt to mislead". That comment was the committee's response to CEGB's argument that forest damage in West Germany is now thought to be caused by ozone, whose formation in the troposphere is stimulated by nitrogen oxides in the presence of sunlight.

Readers of the published report are likely, on reflection, to agree with Marshall that CEGB has been misunderstood, while it also seems that the committee's complaint at the supposed inadequacy of the board's research programme does not assign proper credit for what is being done. Apart from basic research, its board is supporting studies of combustion systems intended to bring reductions of acid emissions of all chemical compositions.

Marshall last week repeated CEGB's position on the steps that could be taken to reduce emissions of sulphur dioxide, saying that "if reducing CEGB sulphur dioxide emissions would solve real environmental problems in the most cost-effective way, we will get on with the job despite the costs". He quoted estimates that the retro-fitting of flue-gas desulphurizing plant to a large generating station might cost £140 million.

According to Marshall's statement last week, the problem of control of nitrogen oxides (NO_x) is different and more difficult. Combustion systems designed to increase thermal efficiency by burning combustible material close to boiler walls will actually increase the production of NO_x , for which reason such a boiler fitted with specially designed burners is to be put in trials beginning in 1986. But CEGB is also planning to carry out trials with a corner-boiler at its Fiddler's Ferry power station. Each experiment will cost about £2 million.

The outcome of the dispute between the nationalized industry and the House of Commons committee is hard to predict. Although the committee seems in part to have reflected a general opinion that "something should be done", it is the British Government and the European

Commission that will eventually determine policy.

Marshall himself, who was made a free-man of the City of London last week, is unlikely to be accused of a breach of parliamentary privilege, even though it is customary for people in his position to leave their defence to the appropriate government department. □

Health advice

Berkeley goes into business

Los Angeles

DEAR SUBSCRIBER ELECT,

AND NOW THERE IS, AT LAST, AN AUTHORITY NEWSLETTER DEALING WITH THE SUBJECT (OF HEALTH AND NUTRITION) THAT IS DESIGNED TO KEEP YOU WELL AND MAKE YOU MORE WELL. HOW AUTHORITY? AS AUTHORITY AS THE UNIVERSITY OF CALIFORNIA, BERKELEY, SCHOOL OF PUBLIC HEALTH CAN MAKE IT.

So begins a solicitation recently mailed to two million households throughout the United States to pay \$15 a year (soon to be \$18) for yet another health publication. But this, according to its promoters, is different.

"Every last word" of each article is approved by the faculty of the Berkeley School of Public Health, says the school's dean, Joyce Lashoff. The faculty's goal in this unusual endeavour, Dr Lashoff says, is to "put current information about health and nutrition in perspective".

In so doing, the School of Public Health stands to make a substantial profit. "They'll do very well", says New York-based publisher Rodney Friedman, who first proposed the idea and raised the venture capital to launch the project. The contract says the school receives 5 per cent of all gross billings, which translates, with regular subscriptions at \$18 per year, into \$75,000 for every 100,000 subscriptions. The publishers' ultimate goal is to attract 250,000 subscribers, when the School of Public Health would earn more than \$180,000 a year for giving its expert opinions and final editing to the newsletter. University of California budgets have suffered cutbacks in recent years, Mr Friedman said, and the public health school will be able to spend the money from its newsletter venture as it sees fit.

The United States market for health and nutrition news seems insatiable. Over 30 magazines and a dozen newsletters publish hundreds of articles each month on how to prevent, cure or cope with illness. Much of the information, according to a recent survey by the American Council on Science and Health, is poor if not dishonest.

The credibility of the Berkeley School

of Public Health stands behind the newsletter, participants say. The eight-page publication, called the *Wellness Newsletter*, aims to give specific advice, written in a short, "palatable" format, on how to improve or enhance health.

A few other universities publish monthly health newsletters, but they are said to be disease-oriented and not written for a broad consumer audience. Nor do individual departments profit so directly from the endeavours. Other academic departments in other institutions may want to keep an eye on the California project as a model for increasing budgets to train graduate students and buy equipment, the objectives which the Berkeley School of Public Health has set itself for the disposal of the income it hopes the project will generate.

Sandra Blakeslee

Chip pirates hit

Washington

SEMICONDUCTOR chip manufacturers last week succeeded in their goal of protecting circuit designs against "chip piracy" — unauthorized sale and distribution of a copied device. Congress finally approved a bill that for the first time grants copyright protection for the masks used to manufacture semiconductor circuits.

The United States dominates the chip supply industry, a world market estimated to be worth \$16,000 million a year in 1983. The manufacturers' complaint has been that a chip costing millions of dollars to develop can be copied photographically and "reverse engineered" for as little as \$50,000, thus denying designers the incentive to innovate. US dominance of the industry has been achieved only through massive research and development efforts: in 1982, average research and development expenditure in the US semiconductor industry amounted to over 10 per cent of sales value, with capital investment over 14 per cent.

Circuit designs, however much careful work they represent, rarely meet the standards of inventiveness needed for patent protection. And, because chips are so easily copied, trade secrecy does not help either. The new bill, the Semiconductor Chip Protection Act, commanded widespread support in Congress and is likely to be signed into law very soon. It provides "mask works" (in whatever physical form) protection against unauthorized copying for 10 years. (Other copyright designs can be protected for up to 100 years.) The bill also breaks new ground in allowing copyright of a utilitarian design; in previous copyright law, only non-functional aspects of design have been covered, a change that appears to have troubled the Copyright Office. The impact on the semiconductor manufacturing industry will be immediate: chip piracy is already big business in some parts of the world.

Tim Beardsley

Israeli aid

Hidden cooperation revealed

Rehovot

AFTER a decade of discreet silence, Israel's programme of scientific and technical cooperation with developing nations has suddenly surfaced. Last month, the International Cooperation Department of the Foreign Ministry announced that Israel had hosted 28,000 trainees from developing countries between 1958 and 1983, and during that same period some 8,500 Israeli experts had worked overseas.

The programme really began in 1957, when Israel helped to create Ghana's Black Star Shipping Line and to organize Ghana's Trade Union Congress. In the years that followed, cooperation flourished on many levels.

During that period, Israel worked not only with national governments but even with some liberation movements, for example training nurses from underground groups in Mozambique and Angola. Although most developing countries cut off diplomatic relations with Israel after the Yom Kippur War in 1973, it now

appears that cooperation has continued with most of them, but quietly.

The emphasis of these programmes has always been on agriculture, but experience has shown that transferring technical know-how is much easier than transferring social forms. The so-called "Rehovot Approach" to integrated rural planning, has, however, proved itself in many developing countries. The aim of this approach, taught so far to 1,000 students from 50 countries at the Rehovot Settlement Study Centre, is to make life in rural areas more attractive so as to stem the flood of country people to urban slums.

The seven-month course, four months of which are spent in Israel, concentrates on the creation of new jobs as well as of a network of economic, social and community services. Comprehensive plans have been prepared for the Lampang Region of Thailand, the Neru Region in central Kenya and several places in Latin America. One group of Israeli experts is now working in Fiji on plans for a multi-purpose rural services centre and the development of rural cooperatives, in collaboration with no fewer than 20 Fijians who have studied in Israel.

In medicine, Israel no longer provides a full-scale six-year course in medicine for students from developing countries, but instead a one-year course in public health and an MA course in community medicine. Much of the effort is concentrated in ophthalmology, as the result of which a dozen African countries have advanced eye clinics. Feasibility studies are first carried out by Israeli physicians; local doctors and nurses are brought to Israel for specialized training and, finally, expatriate Israelis are seconded to this clinic for a couple of years.

As might be expected in a country with enormous financial problems, there are people in Israel who question the wisdom of spending money on cooperation with nations which often do not have diplomatic relations with Israel, and which regularly vote against Israel in the United Nations. Mr Johanan Bein, director of the Foreign Ministry's International Cooperation Department, is philosophical. He says that the total cost of the programme is not large and that more than half of Israel's costs are borne by other countries, mainly in Western Europe, which sub-contract work in fields such as arid-zone agriculture.

Bein also notes that a few African countries, including Zaire and Liberia, have recently re-established diplomatic relations, and that in others without formal links, such as Nigeria, the Ivory Coast and Ghana, Israeli companies are now involved in development programmes much larger than the Israeli Government could have mounted. There are also hopes of a further political payoff, illustrated by the fact that among the 13,000 students from 100

nations who have participated in labour studies programmes run by Israel's General Federation of Labour, there are now 37 secretary-generals of union federations, 300 chairmen or secretary-generals of individual unions, over 150 members of parliament and several dozen ministers, including three prime ministers.

Politics apart, there is also a sense of mission. Forty years ago, chemist Chaim Weizmann, due to become Israel's first president, was corresponding with Maung Kyi (deputy secretary of the Burmese National Planning Department) and with a number of Indians, including Jawaharlal Nehru, about possible scientific cooperation between the Jewish State on the one hand, and India and Burma on the other. Even at the lowest point in relations between Israel and the governments of developing countries in 1973, when most of her long-time friends had turned their backs on her, Golda Meir, then prime minister, said that political disagreements were "no excuse for disowning or belittling . . . an attempt on the part of one country to better human life in other countries".

Nechemia Meyers

Democratic elitism

CHINA, as part of its policy of reversing the anti-intellectual attitudes of the Cultural Revolution, has inaugurated a scheme of state awards for "the promotion of scientific and technological advances". State prizes for science are a feature of most socialist societies, but the Chinese regulations, promulgated last month, contain a unique feature — the decision of the adjudicators is not final.

According to the recommended procedure, the names of the winners chosen by a special examination committee must be published at least three months before the awards are conferred. Complaints against the winners may be lodged within that period. Such complaints will then be investigated by "appraisal units", who will then report back to the examining committee for a final decision. If no complaints are sustained, the winners will receive their prizes.

There are three classes of award, each consisting of a citation and medal together with a sum of money (15,000 yuan — £5,000 — for a first class award, down to 5,000 yuan for a third class). Provision is also made for a "special-class" award, of greater (but unspecified) monetary value, for a "scientific and technological advance that makes special contributions to socialist modernization".

In the case of joint achievements, the cash is to be divided "rationally" according to the contributions of individual team members to the project, with those who made greater contributions getting the greater rewards. "It is impermissible", the regulations warn "to practise egalitarianism."

Vera Rich

Israelis visiting China

Rehovot

AMBIGUITY continues to attend the status of Israelis making scientific visits to the People's Republic of China. So much is clear from the experience of Dr Smil Ruhman, professor at the Weizmann Institute, at the First International Conference on Computers and their Applications in Beijing this summer.

Although Ruhman was an invited speaker listed (with his affiliation) on the programme, he notes that Israel was not listed among the overseas countries participating in a report on the conference published in the *China Daily*. Technically, this may have been correct, because Ruhman entered China on a US passport, having dual nationality.

Others, such as Mordhay Avron, have been less fortunate. Invited by the organizers of the 11th International Sea-weed Symposium to give a talk at Qingdao in June 1983, and listed in the official programme, Avron was told to apply for a visa in some country with which China has diplomatic relations. In the event, he was told by the Chinese Embassy in the United States that a visa could not be issued there and, eventually, by the conference organizer, Dr Tseng, that the invitation to speak was conditional on possession of a visa.

Ironically, Ruhman has returned from China brimming with enthusiasm for the opportunities for collaboration, in which, he says, Israel has some distinctive developments to offer the Chinese. He, like many others, hopes that Chinese policy on passports will change.

Nechemia Meyers

In defence of CERN

SIR — May an observer well acquainted with US and European particle physics comment on the report of social scientists Ben Martin and John Irvine on the achievements of CERN, the European High Energy Laboratory in Geneva (*Nature* 6 September, p.4)? I find this report unfairly biased against CERN.

When European high-energy accelerator research on a large scale began in 1960, CERN was a completely novel social experiment. There was almost no tradition of "big science" in Europe, and no experience of international scientific management. Nevertheless, within less than 24 years, CERN had developed into the world's leading laboratory in this field.

Why then do Martin and Irvine consider CERN's performance disappointing? They concentrate their attention on sensational peak discoveries, in which respect CERN has contributed only in recent decades. But science does not consist of discoveries that make headlines in newspapers. The bulk of science consists of a broad front of painstaking investigations, leading to ever deeper insights. They are the soil from which striking discoveries can develop. In this respect, CERN has made many contributions to which Martin and Irvine give only passing attention.

It is true that some of the peak discoveries, in particular the so-called *c* (charm) and *b* (bottom) quarks, could have been found at the intersecting storage rings (ISR) at CERN. Other "sensational" discoveries, the violation of parity, the Ω -minus and the two types of neutrinos, which CERN is blamed for missing, were made in the United States before or at a time when research had barely started at CERN. But even if one insists on counting the lucky events of that type, CERN did not fare so badly during the second half of its research period. It discovered a new form of radioactivity, the so-called neutral currents, the well-advertised quanta of the weak interaction and probably a sixth quark (*t*, for top) as well.

Martin and Irvine ascribe the latest achievements to a recent change of spirit due to the present director. It is true that Herwig Schopper vigorously supported the last phases of the conversion of the super proton synchrotron (SPS) as a proton-antiproton collider, and the subsequent successful research. However, Martin and Irvine do not seem to appreciate that the conversion of an accelerator and the preparation of such giant experiments take much longer than the three and a half years of Schopper's directorship. The daring spirit and the technical inventiveness that have led to these achievements date from much earlier times. They are a proof of the opportunities given to individual scientists, which Martin and Irvine consider to have been too restricted at CERN. They also show a steady growth of this institution

towards its present world supremacy, that started long ago.

The comparison with Fermilab in the United States is unfairly presented. True enough, several great discoveries were made at that laboratory, such as the existence of a fifth quark. But it was the CERN accelerator that was able to produce sharp and intense muon and neutrino beams with which the so-called structure functions of protons and neutrons were determined with astounding accuracy. Moreover, the important "EMC-effect" was discovered, which has led to a new view of the role of quarks in the atomic nucleus. One of the main purposes of those accelerators was the production of usable secondary muon and neutrino beams, in which CERN was much more successful than Fermilab in spite of the latter's four-year lead. Why do Martin and Irvine not appreciate these successes? Why do they consider them to be dull physics?

CERN's contributions to the physics of nuclear structure are almost completely neglected in Martin and Irvine's report. CERN has the most advanced isotope separation device (Isolde) attached to its synchro-cyclotron, where many new unstable nuclei have been discovered and studied. Recently, a low-energy source for antiprotons was constructed (LEAR) which provides unique opportunities for the study of antiproton interactions.

The success of CERN is based to a large extent upon a circumstance that has been a surprise for people who have observed the development of physics in the past half century. The United States was assumed to be superior in engineering and instrumentation, as demonstrated by the development in the 1930s of cyclotrons, synchrotrons, linear accelerators, bubble chambers and so on. But the quality of the engineer-physicists at CERN turned out to be at least as high. They succeeded in constructing accelerators of unusual reliability and flexibility under the leadership of the late Sir John Adams. Some have ascribed this to over-conservative design, but it helped greatly in the exploitation of the machines. Thus it allowed the luminosity of the intersecting storage rings to reach more than ten times the design value, and it facilitated the production of the intense muon and neutrino beams. Finally, it made possible the fast conversion of the 400 GeV accelerator into a proton-antiproton collider, the success of which hung on one of the imaginative devices invented and implemented at CERN by Simon Van der Meer — the so-called stochastic cooling of the antiproton beam, destined to be used in future colliders everywhere in the world.

Furthermore, CERN engineer-physicists led by Kjell Johnson constructed the intersecting storage rings at a time when laboratories in the United States did not dare to take on this difficult task. CERN

designed and built the first two colliders for protons, the ISR and the proton-antiproton collider in the SPS tunnel. The effect has been to pioneer a new style of experimentation which has now been taken up by other centres of particle research all over the world. CERN continues the tradition with the large electron-positron collider LEP.

The Martin-Irvine report does not make much of these achievements and also all but ignores the many new ideas for instrumentation spawned at CERN, such as the multi-wire proportional chamber and the vacuum ultraviolet imaging device. Both innovations are not only significant advances in detecting particles, but have important applications in medicine and in other sciences.

High-energy physics is an international endeavour, more so than most other scientific activities. There is a constant exchange of physicists and engineers between CERN, the United States and other countries. It does not make much sense to consider progress in this field as a competition between two continents. It is a collective achievement of all participating nations. The CERN effort has contributed essential ingredients. Particle physics would not be what it is without CERN's impetus, without its technical innovations and without its studies and discoveries, even if some of them have not made newspaper headlines.

Last and by no means least, CERN represents the United States of Europe in fundamental physics. It became an active symbol of the spirit of European unity. That is no mean achievement. It serves as the University of Europe for high-energy physics, and brings together the many individual universities in Europe in scientific collaborations of unprecedented magnitude and complexity often involving also universities and institutes of the United States, Eastern Europe and Asia.

In spite of the obvious difficulties which such international collaborations entail, CERN has developed into the foremost high-energy laboratory of the world, as is strikingly confirmed by the recent US panel report on New Facilities for the US High Energy Physics Program, in which it was stated that "the European facilities frequently provide a better level of support and/or a larger number of opportunities than the American equivalents".

How can such an institution be given "Poor Marks for Enterprise"?

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Victor Weisskopf was director of CERN from 1961 to 1965.

SIR — Robert Walgate's enthusiasm (see *Nature* 6 September, p.4) for the assessment of CERN carried out by two social scientists from Sussex, Irvine and Martin, is misplaced. Most would agree with one conclusion reached by Martin and Irvine,

that since the end of the 1960s CERN has been either the leading or next to leading laboratory in the world of particle physics. But their claims to have developed a powerful new tool of assessment in scientific research are exaggerated and their use of it is misleading.

Originally, Martin and Irvine advertised their method as one of "converging partial indicators", based on the questionable assumption that if a variety of uncertain measures appear to agree, the result is reliable. Having applied this method to CERN and found that the indicators do not converge, they nonetheless continue by "interpreting" the non-convergence. Here they rely on an opinion poll of physicists, so the new method boils down to a peer review interpreted by social scientists.

The authors base their main conclusions on a selection of so-called "crucial" experiments. This is hardly the way a serious history of the extraordinary progress in particle physics over the past ten or fifteen years will eventually be written. Such an approach traps the unwary into supposing all other results are negligible, and is a travesty of the basis on which scientific understanding advances. CERN is given credit for the discovery of the weak neutral current, which opened the door for the Glashow-Salam-Weinberg unified theory of the electromagnetic and weak forces and set the stage for much of what has followed. But this discovery did not win a place in the "top eleven" selected by citation analysis.

The method clearly fails seriously in not giving proper weight to several other CERN results of fundamental and lasting importance. For example, experiments at the intersecting storage rings discovered that the strong interactions of the proton are due to point-like constituents; deep inelastic neutrino experiments showed that the weak interactions of the proton also involve point-like "partons" and, when put together with the electron scattering results from the electron linear accelerator (SLAC) at Stanford, demonstrated that the partons are quarks. High-energy neutrino experiments found departures from point-like scattering of a kind attributable to the effects of the quark-quark forces, providing early support for quantum chromodynamics, the now established theory of the strong interaction.

Nevertheless, it is an accepted view that over the decade 1969 to 1978, US laboratories would have appeared more often in a list of top experiments than Europe. Why? Because Martin and Irvine have set themselves the objective of judging CERN, they have no device but to attribute this difference in performance to some inadequacy of managements and physicists at CERN. Unsupported by evidence, they even attribute what they regard as CERN's poorer performance to its being an international laboratory with scientific advisory committees accountable to national interests. Anyone with the slightest

acquaintance with the ways CERN's scientific programme is handled knows there is no such interference; in making this inference Martin and Irvine display such a total ignorance of how CERN actually works that it must invalidate any other conclusions they reach.

With all their great effort of hindsight, Martin and Irvine are yet blind to the single most significant factor in the Europe-United States comparison in the 1970s: the electron-positron collider (SPEAR) at Stanford, together with SLAC, was responsible for the major part of the difference in score on which they hang their case. All will agree that SPEAR was, supremely, the best type of machine for the physics of the 1970s; once this factor is allowed for, the differences are not significant.

No doubt, in retrospect, some decisions of the CERN management and actions of some physicists if differently made could have led to greater success. But this can be said of most scientific endeavours — including those in the United States. What Irvine and Martin seem unable to understand, and what makes their analysis so misleading, is that in research of this nature, until the experiments are done, no one knows which is the best choice. This is why the "methodology" of Martin and Irvine could not have predicted the remarkable success of Stanford in the 1970s.

The authors conclude that after the 1970s, CERN's performance has improved. We are given no "explanation" for this, which the analysis of the preceding years would not have foretold. Their naivety makes any views they express irrelevant to the assessment of any future enterprise such as LEP.

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Mental arithmetic

SIR — I was surprised to read in David Singmaster's review¹ of Steven B. Smith's book, *The Great Mental Calculators*, that the mental calculation of the square root of 51 is considered to be so difficult.

I have just calculated the answer to be 7.14142842854216; the last two digits should be 85 I believe. To perform this calculation I have to write down the number (in fact 510,000,000,000 is easier, giving the required answer $\times 10^5$), and also write down and see my growing answer. I have been able to do this for quite some time, but have not developed the capacity, it being of somewhat limited use!

To calculate 1/97 to 96 decimal places, another example given by Dr Singmaster, seems quite easy, and I can multiply three-digit numbers in my head (again, provided they are written down), with a fairly high success rate. From these examples, I could match Alexander Aitken, one of the

prodigies cited in the review, but not George Bidder. Indeed I have never thought in terms of logs, as did Bidder — Taylor or Maclaurin series presumably? However I would think that compound interest calculations could be achieved fairly easily by use of suitable binomial coefficients.

As a postdoctoral biochemist I rarely have any call to use such mathematical abilities. But I am astounded that they are thought to be so rare.

BRIAN COOPER

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1. *Nature* 310, 79 (1984).

Animal rights

SIR — In his article "Animal rights lobby cashes in" (*Nature* 13 September p.95) Marcus Chown points out that it is more than a year since the Government White Paper on animal experiments appeared. Neither in your article nor in recent articles and letters in *The Times* is mention made of certain wording relating to pain which I believe may be responsible for misunderstanding and anxiety. Mr Chown quotes the present rule "if an animal is found to be suffering severe pain which is likely to endure, it shall at once be killed". That is not unfortunately the whole story. Every licence to experiment on living animals carries a list of conditions. The passage quoted is from paragraph 3(b) of these conditions. 3(a) states that if an animal is suffering pain which is either severe or is likely to endure, and *if the main result of the experiment has been attained*, the animal shall forthwith be painlessly killed (*italics mine*). It is this which gives rise to problems and leads those of a suspicious mind to say that there is always a let-out. An experimenter faced with the loss of valuable experimental material might be tempted to hang on for a few more days until the experiment has been completed, with consequent suffering. This wording must be clarified. An important purpose of future legislation must be to allay public suspicion or disquiet and there must be no suggestion of an easy escape. Paragraph 3(c) of the conditions states that if an Inspector believes that an animal is suffering "considerable" pain he can direct it to be painlessly killed. We thus have pain described as "severe" and "considerable". Since it is impossible to give a clear definition of pain in animals, these terms are of doubtful value. Nevertheless the more moderate animal welfare organizations are right on insisting on some clarification of the "pain condition" and some attempt to balance possible suffering against possible benefits is not unreasonable.

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Prizes (at last) for immunology

This week's Nobel prize comes less than 10 years after monoclonal antibodies, already produced commercially as diagnostic reagents. But in that decade, immunology has been transformed.

SOME Nobel prizes are inevitable, the only question being when the nomination will rise to the top of the pile. This year's prize for César Milstein must have been close to the top for at least three years. Nor does it come as any great surprise that Georges Köhler is the second of the three winners of this year's prize for Physiology and Medicine, for he and Milstein jointly discovered the principle of producing monoclonal antibodies. Less predictable is the third winner, Niels Jerne. But to immunologists, Jerne is the great man of influential ideas.

Köhler and Milstein published their paper on "Continuous cultures of fused cells secreting antibody of predefined specificity" in August 1975 (*Nature* **256**, 495). It described how the fusion of a single lymphocyte-secreting antibody with a single bone marrow tumour (myeloma) cell resulted in a hybrid cell with the antibody-producing capacity of the lymphocyte and the immortality of the malignant cell. Their antibody-producing cells came from the spleens of mice immunized with red blood cells from sheep, so that the fused cell lines (hybridomas) each secreted an antibody directed against the sheep cells. Köhler and Milstein emphasized that they could not tell whether similar results would be obtained with other antigens but, obviously believing they could, finished their paper by saying: "It is possible to hybridize antibody-producing cells from different origins. Such cells can be grown *in vitro* in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use."

They were right. Specific antibodies, now known as monoclonal antibodies, are an essential tool of much biomedical research and are of great commercial and medical value. The therapeutic use of monoclonal antibodies is still at an early stage, but they are already invaluable aids to diagnosis and screening. A good example is in blood grouping, where hybridomas established by Milstein are now the basis for the production of monoclonal antibodies on a sufficient scale to replace human sera in ABO blood typing in the United Kingdom.

César Milstein was born in Argentina in 1927, and was on the staff of the Instituto Nacional de Microbiología in Buenos Aires until 1963, taking time out to obtain a PhD at Cambridge in 1960 as a British Council Fellow. In 1963, he left Argentina to join the staff of the Medical Research Council Laboratory of Molecular Biology in Cambridge, where he has been ever

since. He now heads the Protein and Nucleic Acid Chemistry division, which he previously co-directed with Dr Fred Sanger — twice a Nobel Laureate, now retired.

Milstein has always worked on the structure, genetics and evolution of antibody molecules. Perhaps the best known of his "pre-monoclonal" papers is that in *Nature New Biology* **239**, 117; 1972, which demonstrated that the light chain of antibodies is first produced as a larger precursor molecule, whose extra sequence serves as a signal for the process of attachment to, and secretion through, the lymphocyte membrane, during which it is cleaved off. This was probably the first firm evidence to support the signal sequence hypothesis of Blobel and Sabatini.

In 1974, Georges Köhler, then a 27-year-old West German at the Basel Institute of Immunology, obtained an EMBO grant to work at Cambridge with Milstein. His chief interest was the development of antibody-producing cell lines to allow the analysis of the rates of mutation of their antibody genes and so to study the role of somatic mutation in the generation of antibody diversity. It was not by design that monoclonal technology emerged from this research, and the relative contributions of Köhler and Milstein to the discovery is a matter of controversy. The pair have shared some minor prizes, but both the Wolf prize (an Israeli's answer to Nobel) in medicine and the Sloan prize of the General Motors Cancer Research Foundation went to Milstein alone. The Nobel committee has obviously decided that Köhler played a substantial part in the discovery. Köhler returned to the Basel institute in 1976, becoming a permanent member in 1980; he has maintained his interest in the mechanism of antibody diversity. Next March he becomes co-director of the Max Planck Institute of Immunology in Freiburg.

When Köhler returned to Basel it was still under the direction of Niels Jerne, the founding director of the Basel Institute of Immunology, from which he retired in 1980. Jerne was born in Denmark in 1911, started life as a student of physics but soon switched to medicine. From 1943 to 1956 he was at the Danish State Serum Institute and there began his studies on antibodies. He had spells of a few years in Geneva (for the university and World Health Organization), as a visitor at the California Institute of Technology, at the University

of Pittsburgh and as director of the Paul Ehrlich Institute in Frankfurt. Technically, his most important paper of that period is probably the description of the plaque-forming assay (*Science* **140**, 405; 1963), a test that allows the quantitation of antibody-producing cells of a particular specificity among a mixed population.

In 1969 when Hoffmann-La Roche founded the Basel Institute of Immunology, Jerne became its first director. During his tenure as director, he became ever more widely acknowledged as the father of several influential, if not always correct, ideas in immunology. In particular he was involved in the theory of antibody selection by antigen — the doctrine that antigens do not instruct the immune system to manufacture new antibodies, but rather select the relevant antibody-producing cell from a pre-existing pool.

Jerne's network theory of immunology, an outcome of his analytical approach to a subject bedevilled by murky observations and operational definitions, was at its most influential about a decade ago. It was based on the fact that antibodies can themselves serve as antigens, Jerne postulated that any antigen would set off a train of events, with each antibody acting also as an antigen and thus linking cells of the immune system in a network since their antigen-binding sites were almost certain to recognize one of the antibodies produced in the course of the response. But in spite of strong experimental evidence in support of the network theory, it remains difficult to determine whether the regulation of most immune responses is substantially controlled in this way.

This year's prizes will be thought well deserved. They will be seen as further justification of the British Medical Research Council's support of basic research at its Laboratory for Molecular Biology in Cambridge. Milstein joins Aaron Klug, Francis Crick, James Watson, Max Perutz and John Kendrew as well as Sanger (twice), whose work at Cambridge brought them a trip to Stockholm. The prizes will also no doubt be seen in certain quarters as a justification of Hoffmann-La Roche's wisdom in founding a basic research institute. But above all this year's Physiology and Medicine prize demonstrates how theory, experiment, curiosity and serendipity can produce not only great scientific insight but what is certain to turn out to be tremendous benefits to mankind.

Peter Newmark

Mineralogy

A continent in a grain of sand

from Bruce W. Sellwood

FOR well over sixty years the abundance, identities and assemblages of minerals in sandstones have been used in the determination of sediment provenance in order to reconstruct the geography, climate and geology of sediment source terrains of the past. Recently, it has been realized that sandstone composition is to a large degree also governed by plate tectonics. Provenance studies of modern sedimentary systems have previously been carried out on the scale of individual basins, but not for a whole continent. Now on page 645 of this issue, Potter describes the mineralogy of sands from the beaches of South America, and interprets it in terms of the palaeotectonics of the continent.

In ancient systems, the petrography of sandstones has been used in the interpretation of the geotectonic regimes of depositional basins, such as a passive continental margin or a fore-arc basin, and of source areas, despite the fact that sand is recycled from older sandstones. Geotectonic interpretations rely heavily on observations and assumptions of the probable composition of typical terrestrial terrains. It is argued that quartz-rich sediments are associated typically with passive continental margins and that quartz-poor sediments are mostly of volcanic origin in magmatic arcs. Sediments of intermediate quartz content are generally believed to be associated with active continental margins and orogenic belts while arkosic (feldspar-rich) sands are often derived from uplifted basement rocks adjacent to rifts and wrench faults. Other factors, such as climate and the length and vigour of streams, are considered to tune the ultimate composition of deposited sands.

The validity of these palaeotectonic models can be tested only in terms of large-scale modern investigations and this is the significance of Potter's study. He chose South America because it has a well-developed active margin on its west coast, a passive margin on its east coast, with transcurrent motions affecting only the Caribbean border zones. Although lacking significant continental glaciation it exhibits major climatic belts lying transverse to the Andes. Potter's is the first report on the petrography of sands from within the same environmental regime (beaches) around an entire continent and thus is highly significant, even though the results come from only about 200 samples.

Much of the sediment arriving on continental beaches is derived from rivers and Potter argues that its mineralogy is likely to have been extensively modified by abrasion. Furthermore, if the mineralogy of beach sands closely relates to the

tectonic setting of adjacent land areas, then that of the local river sediments is likely to be even more closely related. The results of the study confirm the predictions of earlier workers (for example, Dickinson, W.F.R. & Suczek, C.A. *Bull. Am. Ass. petrol. Geol.* **63**, 2164; 1979). On its leading margin (the Pacific coast), quartz comprises only 19 per cent of the mineral and so is subordinate to rock fragments and feldspar, while on the trailing margin (Atlantic coast) it comprises, on average, 96 per cent of the mineral, even where mountains of Precambrian crystalline rocks border the coastline.

Some anomalous patterns exist, however, indicating that results from ancient sequences must be interpreted with some caution. Although sited on a trailing margin, the Argentine coast from Rio de la Plata to Tierra del Fuego has an Andean (leading-margin) composition, being dominated by volcanic rock fragments. This is partly because Patagonia is too narrow to allow abrasive processes to be completed and partly because the coastal bedrock is itself immature young sediments recently derived from the rising Andes.

This work thus confirms the view that the detrital mineralogy of ancient sand-

stones may be used successfully in the reconstruction of palaeoplate settings.

Although the predicted patterns of mineralogical provinces are generally confirmed in the study, further questions need to be answered. For example, what subtle effects does climate have on the rate of degradation of certain types of rock and mineral species during transport? In what way does the interaction between relief, transport distance and rainfall favour either the elimination or preservation of unstable grains? And to what extent do the boundaries between mineralogical provinces coincide with major physiographical features such as large river mouths?

Potter's study is merely a first step, doing for grains on a continental scale what clay mineralogists have already done for mudrocks on a global scale (Griffin, G.M., Windom, H. & Goldberg, E.D. *Deep Sea Res.* **15**, 433; 1968). As Potter observes, if his models stand up to further scrutiny, the next stage will be deduction of sand composition over all types of environment of an entire continent (or palaeocontinent). Because the depositional maturity of sediments is a key factor in their behaviour during burial, predictive models based on studies such as Potter's bear directly on the assessment of regional porosity and permeability trends in hydrocarbon reservoir rocks. □

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Astronomy

Nemesis for Nemesis?

from Mark E. Bailey

LAST spring, great interest and excitement were generated by the idea that a hypothetical companion star of the Sun, called Nemesis by some, could trigger the periodic sequence of extinction events found in the fossil record¹⁻⁵. Now, only six months later, another collection of papers on the same subject (see pages 635-642) gives what amounts to a considered reflection on the model.

Interest in Nemesis began about a year ago⁴, following the claim by Raup and Sepkoski to have identified a regular 26-Myr periodicity in the fossil record, with a confidence level of better than 99 per cent. The evidence that the sequence of events was periodic was further strengthened by an independent analysis of the cratering record³, which produced a period and phase for the cyclicity agreeing with that of the fossil record. These arguments for strict periodicity in the geological record can be challenged at a number of points⁵, although the independent evidence for rough periodicity as, for example, in the sense that the solar sunspot cycle is 'periodic', is probably less easily denied⁶⁻⁸.

But if the cratering and extinction record is strictly periodic, which will be settled only by further detailed research on the geological front, it is clearly important to devise explanations, and this is the spirit in which the Nemesis proposal was made. Thus, it was argued, the orbital period of the hypothetical companion should give the strict periodicity required, while its gravitational influence on the comets in a hypothetical dense inner core of the Oort cloud might produce periodic comet showers in the manner outlined by Hills⁹. The further possibility that Nemesis (or Planet X) might show up in the Infrared Astronomical Satellite data also gave piquancy and a certain urgency to the debate.

In the papers published in this issue of *Nature*, the Nemesis proposal is extended and shown, in fact, to be quite incapable of producing the strictly periodic sequence of extinction events for which it was originally designed. The stability of the orbit has been investigated by Torbett and Smoluchowski¹⁰, whereas Hills¹¹ and Hut¹² show in particular that when stellar pertur-

bations and the overall tidal field of the Galaxy are included, the orbital period should drift towards larger values by about 15 per cent or more over the 250-Myr time scale considered by Raup and Sepkoski. The probably dominant effects of giant molecular cloud perturbations would further reinforce this conclusion, and cast serious doubt on the likely survival of such a solar companion for the age of the Solar System^{10,13}. Of course, it is conceivable that the star might have been recently captured or scattered to its present orbit from a more tightly bound state with much shorter period, but these suggestions beg many questions. In any case, as shown by Hut¹², it appears that the Nemesis proposal — if correct — does not predict a constant interval between extinctions. A re-analysis of the fossil record may agree with this new prediction², but this has not yet been carried out.

The alternative, that extinctions are quasi-periodic, was explored last April in two papers^{14,15} and is now reviewed in detail by Clube and Napier¹³, who argue strongly against the Nemesis proposal and come down in favour of a galactic connection, as they have advocated for some time^{6,7,16}.

In view of these developments, one might possibly be excused for regarding this episode of hypothesis followed by near-retraction as yet another example of the explosion in the volume of the literature, and of the over-rapid publication of speculative ideas. Previous commentaries^{4,5} already hinted at this, and it is therefore perhaps not wholly out of place to consider the point further. Although some cynicism is doubtless justified and can easily be understood, to hold such a view exclusively is, in my opinion, to miss the point. It is often the case in astronomy, and no doubt other subjects, that the various pieces of a puzzle can be assembled in a number of ways and still explain the same data. Indeed, it is an important part of a theorist's job to do just that, and provided the proposed hypotheses take all the facts into account and are not obviously so contrived as to be subject to Occam's razor, the more possibilities the better. This in spite of the reality that no more than one can be true. Science often progress by mistakes and it is therefore too easy simply to dismiss speculative suggestions as a kind of non-science.

In the present case, the argument for Nemesis causing mass extinctions is indeed highly speculative since it assumes the existence of the star, the presence of a dense inner core to the Oort cloud, the regularity of the extinction cycles and the possibility that impacts trigger extinctions. Yet whatever the final decision about the existence of the star, the benefits of the suggestion have already outweighed any temporary nuisance or adverse reaction it may originally have caused. For example, the shape of the Oort cloud and the stability of comet-like orbits under the perturbing action of

passing stars, gas clouds and the Galaxy, are crucial to an understanding of cometary origins. Moreover, the papers now published have a clear connection with studies of binary stars and of their long-term orbital stability, while the connection between these kinds of problem and studies of open star clusters has already been noted¹⁷.

On the geological front, I am less qualified to judge, but if the Nemesis saga has served only to increase general awareness that cometary impacts may frequently occur, then it will have been beneficial. Ten years ago the idea was hardly considered, and even last year, at the Brighton meeting of the British Association for the Advancement of Science, it was dismissed. Yet the most conservative Oort cloud model predicts some cometary impacts, and presumably they do have some influence worth exploring.

Finally, if the extinction sequences should somehow be shown to be strictly periodic, it may be worth noting that there

is already a new variant on the Nemesis theme¹⁸ — in which comet showers are still predicted to be periodic. To mix two metaphors, though there may be smoke without fire, perhaps even the darkest clouds have silver linings. □

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Behavioural ecology

Optimal foraging theory tested

from Jared M. Diamond

A KEY postulate of behavioural ecology is that animals practise 'optimal foraging'. That is, animals forage for food in a way that maximizes some function such as the rate of energy gain^{1,2}. A paper by Carpenter *et al.* describes an ingenious experiment designed to test the theory of optimal foraging³.

Consider the territory size of an animal attempting to maximize its daily net energy gain. Optimal foraging theory argues that a territory yields benefits (especially food resources), which increase in proportion to territory size up to a ceiling set by an individual's food-processing abilities or time constraints. An increase in territory size also incurs increased costs of travel time and territorial defence against intruders. Supposedly, individual energy-maximizing animals can both assess and achieve an optimal territory size at which the difference between the benefits and costs represents a maximal rate of energy intake.

While much animal behaviour is roughly consistent with this view, it has proved very difficult to devise quantitative tests of the optimality principle in the field. A suitable test would require measuring an animal's rate of net energy intake (approximated by its rate of weight gain) as a function of the parameter putatively optimized (for example, territory size). One could then examine whether the rate of weight gain does peak at intermediate territory sizes, and whether individuals adjust territory size towards this optimal value.

How could one perform such a test? Periodically capturing a wild animal to

weigh it has the obvious drawbacks that one can rarely count on capturing an individual at fixed intervals, and the disturbance associated with capture is likely to influence the results. The ideal solution would be to induce an animal to weigh itself repeatedly. It is this task of persuasion that has recently been achieved by F. Lynn Carpenter, David C. Paton and Mark A. Hixon, working with rufous hummingbirds (*Selasphorus rufus*) of western North America³.

Like most other migratory hummingbird species, the rufous faces severe energetics problems. In July and August of each year the birds migrate at least 3,000 km south, along mountain ranges, from their breeding grounds (Oregon to Alaska) to their wintering grounds in southern Mexico⁴. To fuel their flight, these 3-g birds stop en route, defend feeding territories and gain at least 1.5 g of fat in 1–2 weeks before resuming migration. At this season, migration in the mountains is risky: rain, hail and wind often prevent feeding, destroy the flowers that serve as nectar sources and even endanger the birds themselves. Thus, individuals should be selected to make few stops, and to fatten up quickly and maximize daily net energy gain while on stopover, so as to complete the migration as quickly as possible.

In order to obtain exclusive access to the nectar of flowers^{5,6}, hummingbirds engage in two types of fierce competition for territories. First, territory-owning hummingbirds spend much time and energy driving off intruding hummingbirds and sometimes bees, hawkmoths and

butterflies which drink the same nectar ('interference competition')⁷. Second, territory holders start off each day by depleting the nectar in the flowers at the periphery of their territories⁸. Since intruders mainly try to rob peripheral flowers and return to sites where they were previously successful, early-morning peripheral foraging by the territory owner spoils the territory for intruders ('exploitation competition').

In 1979, Carpenter and her colleagues tested a model of optimal territory size⁹ by covering half the flowers in a hummingbird's territory with plastic bags¹⁰. They found that within one day the bird increases its territory size to maintain the original number of defended flowers, and then abandons the extra area when the bagged flowers are unbagged on the next day. This experiment shows that the benefit gained from territory size is control of food, and that hummingbirds are capable of measuring their nectar intake or nectar resources. The experiment does not assess, however, whether the preferred flower supply is optimal, as defined by maximization of weight gain.

In 1980 the following direct test was devised. A hummingbird spends about 75 per cent of each hour quietly perched on one or two preferred twigs while clearing its crop of nectar between foraging bouts. Carpenter and colleagues replaced these twigs with a Pesola spring balance, modified to carry a perch on top and to function by being depressed from above rather than stretched from below. The bird accepted this perch, thereby enabling Carpenter *et al.* to sit with a telescope outside the territory boundaries and periodically read off the bird's weight to ± 50 mg. In 1982 the spring balance was exchanged for a Mettler electronic balance whose pan had been replaced by a perch. With the resulting weighing accuracy of ± 10 mg, the biologists could detect a hummingbird's weight gain in a single foraging bout or its weight loss on defaecation.

As an example of the results, consider

bird WP, who was first observed on 23 July as a lean individual weighing 3.1 g. On 27 and 28 July WP had a relatively small territory containing 1,970 flowers and gained weight at the low rate of 0.10–0.15 g per day. On 29 July WP nearly doubled its territory size to 3,320 flowers and increased its weight gain to 0.25 g per day. The following day it relinquished half of the extra flowers to achieve its maximal rate of weight gain of 0.35 g per day, and it maintained this territory for another day. It thus reached a weight of 4.6 g by the morning of 1 August and took off on migration.

The results with WP and other rufous hummingbirds reveal that the birds adjust their territory size by trial and error until they have maximized the rate of weight gain³. Territories that are either too large or too small yield reduced rates. Hence, this system supports two basic postulates of optimal foraging theory: the birds can detect changes in energy intake, or a correlate of it, and they apparently adjust territory size to maximize energy intake. More generally, however, this study offers a method of repeatedly weighing a wild animal without disturbance. With further ingenuity the field balance technique could be applied to other animal species that periodically return to one or a few preferred sites. Such regular weighings of wild animals could elucidate behavioural, physiological and ecological problems, such as ones related to foraging, water loss and reproduction. The new method may provide a breakthrough in field biology. □

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provide the strongest support for the autocrine theory of malignancy. High-affinity receptors for PDGF are confined to a well-defined subset of animal tissues — only fibroblasts, glia and arterial smooth muscle cells display them, and only these cells show a growth requirement for PDGF in culture. Last year, the *c-sis* oncogene was shown to direct synthesis of a functional PDGF subunit. As expression of this gene is (almost) exclusively confined to cells derived from connective tissue neoplasms, it can be concluded that synthesis of the *sis* protein (PDGF) *in vivo* provides a selective advantage only to cells that express PDGF receptors.

Seifert *et al.* report that cultured aortic smooth muscle cells isolated from 13–18-day-old rat pups secrete a growth factor that competes with authentic human PDGF for binding to fibroblasts. This cellular PDGF-like material (which they term PDGF-c) is recognized by anti-PDGF immunoglobulin and is mitogenic for 3T3 cells. Comparable smooth muscle cells isolated from adult rats secrete little, if any, PDGF-c. The inability of adult cells to generate PDGF-c does not merely reflect the passage of time or cell divisions, because the pup smooth muscle cells continue to secrete PDGF-c during 20 passages in culture and over a 6-month interval. The authors conclude that synthesis of PDGF-c is developmentally regulated. They speculate that the substantial increase in both the number of smooth muscle cells and the thickness of the connective tissue matrix of the rat aorta, that occurs within the first three months, reflects an automitogenic response to PDGF-c.

Synthesis and secretion of PDGF-c by pup smooth muscle draws attention to a broader body of literature on the production of growth factors either by normal diploid cell cultures derived from embryos or by embryonal carcinoma cell lines. Substances identical to, or closely related to, nerve growth factor, transforming growth factors and colony-stimulating factor are synthesized by these cells^{4–6}. Two recent studies echo the developmental control observed by Seifert *et al.* First, Nissley, Rechler and their co-workers have shown that diploid fibroblast cultures derived from neonatal rats secrete the neutral form of insulin-like growth factor (IGF-II) which seems to modulate growth *in utero*. Comparable cultures established from adult rats secrete the basic form (IGF-I) which is the dominant factor in skeletal development of juveniles and adults⁷. Second, Clemmons has noted that while fetal fibroblast cultures secrete a functional homologue of PDGF, comparable cultures derived from human donors aged three or more do not⁸. Though Clemmons did not have the PDGF radio-receptor assay at his disposal, it seems plausible that the mitogen he observed is the PDGF-c described by Seifert *et al.* The plethora of growth factors secreted by embryonic cells, the developmental control

Developmental biology

Autocrine control of growth?

from C.D. Stiles

In the 'autocrine growth' scenario for malignant transformation, popularized by Todaro, DeLarco and others¹, cancer is initiated when a cell begins to produce a growth factor for which it has receptors and to which it is responsive. When this factor binds to receptors on the cell from which it has been secreted, a self-perpetuating automitogenic response is triggered. Within this context, autocrine growth is seen as a perversion of normal information flow, it being tacitly understood that growth factors, like conventional hormones, function normally in

the paracrine or endocrine modes — that is, they communicate between heterologous tissues and are synthesized at some distance from the target cell. A letter in this issue of *Nature* challenges this precept; in it, Seifert *et al.* describe the developmentally regulated synthesis of a growth factor that may normally function in the autocrine mode².

Ironically, platelet-derived growth factor (PDGF) serves as the point of departure for the studies of Seifert *et al.* It is recent insights into the molecular biology of this molecule (reviewed in ref. 3) that

of their production and the early appearance of growth factor receptors in embryogenesis⁹ support a popular prejudice that the serum growth factors, scrutinized so thoroughly within the context of tumour biology, may play pivotal roles in developmental growth and morphogenesis.

The new twist provided by Seifert *et al.* is the suggestion that 'embryo-derived growth factors' function normally in the autocrine mode. On first consideration the concept is aesthetically distasteful. Why should a developing tissue secrete a growth factor to stimulate its own proliferation? Surely the process could be controlled more stringently from within the cell at some point subsequent to the events governed by growth factors and their receptors. A teleological rationale may be contained within the biological need to amplify weak chemical signals — the *raison d'être* of membrane-bound receptors for hormones and growth factors. It is worth noting that normal embryo fibroblast cultures typically require less serum for growth than do established lines of the 3T3 genre, an observation consistent with the view that embryonic cultures respond to the growth factors they produce. Furthermore, another growth factor, interleukin-2, seems normally to function in the autocrine mode¹⁰.

The interface between growth factors

and development remains even less than tenuous. Nonetheless, if recent observations can be extended, the implications are wide indeed. Growth factor receptors and other components of the 'mitogenic cascade' may be vestiges of an automitogenic apparatus used in development. By switching off the gene encoding an automitogen and activating the same gene at some distal site, the developmental machinery may be neatly converted to the paracrine mode. In this state, it could promote cell proliferation 'on demand' during adult life in, for example, wound healing, erythropoiesis and immunogenesis. Switching errors in adult life would manifest themselves as proliferative disorders — keloids, psoriasis, atherosclerosis and, of course, neoplasia. □

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Biophysics

Many guises in Bristol

from Maxine Clarke

There probably never was an adequate definition of biophysics. Perhaps there never will be. That, at least, seemed to be one inevitable conclusion of the 8th International Congress of Biophysics held in Bristol, 29 July — 4 August, at which it became clearer than ever just how many biological questions have recently been annexed by biophysicists.

For example, T. Wiesel (Rockefeller University) presented one classic physiological study in the guise of biophysics in reporting how electron microscopy has been used to demonstrate axonal spread of peroxidase-stained neurones throughout several hierarchical levels of organisation in the visual cortex. Physiological experiments on monkey photoreceptors (D. Baylor, Stanford University) show that both rods and cones have identical properties to those deduced by psychophysical studies in man. Many other biological questions, from how ion channels are gated to how microtubules cause cilia to beat, were similarly addressed in biophysical fashion.

To anyone with a primary interest in the structure of cells and organelles, it was very obvious that improvements in electron microscopy and synchrotron radiation measurements had contributed greatly to

progress since the 7th congress, three years ago. An optical diffraction pattern taken from an electron microscope image contains information about the phases as well as the amplitudes of the reflections. This inherent advantage of electron microscopy over X-ray diffraction techniques has been limited by problems in the resolving power of the method, but the use of low electron dose to eliminate beam damage to the specimen and the development of cryomethods which do not require staining of the specimen are solving some of the problems. Such improvements enabled E. Kellenberger (University of Basel) to show that the bacterial cell envelope has a periplasmic gel of regular thickness between the inner and outer membranes, with the nucleoids (bacterial DNA) much freer than originally thought. The dependence of nucleoid structure on environmental conditions is now being measured by a freeze-substitution technique. J. L. Carrascosa (Universidad Autónoma de Madrid) produced a 2.2 nm-resolution three-dimensional image of the head-to-tail connection (neck region) of the 029 bacteriophage by tilting a two-dimensional hexagonal array of necks and performing a Fourier analysis of differently angled views of the section. The inner region of the neck

was identified as the part interacting with the DNA; it has 6-fold symmetry and the proteins constituting this and the 12-fold outer neck region have been assigned.

Improvements in synchrotron radiation techniques have enabled some of the predictions of the crossbridge theory of muscle contraction to be confirmed for the first time. A two-dimensional detector allows a difference pattern between relaxed and contracting muscle to be produced. H. Huxley (MRC laboratory of Molecular Biology, Cambridge) reported an increase of the actin (thin filament) reflections on contraction; this result is the necessary outcome of the attachment of crossbridges from the thick to the thin filaments but had not been visualized previously. His group has also found that the part of the diffraction pattern that arises from the calcium-binding proteins changes before the part that is related to the crossbridges. This confirms the hypothesis that calcium binding has a structural consequence which must express itself in the muscle filaments before the crossbridges can produce force. In a different approach to elucidating the mechanism of contraction, Y. Goldman (University of Pennsylvania) described an ambitious attempt to link the biochemical rate constants of the muscle's ATPase, derived from solution studies of muscle proteins, with the force-producing properties of the fibres. Caged ATP, an inert molecule which can be photolysed to ATP with UV light after its infusion into a muscle fibre, is used in this approach. Several of the kinetically intermediate reactions in the ATPase in muscle fibres are similar to those in solution, so that the particular step in the biochemical pathway affected by force can now be sought.

Innumerable other topics, ranging from atomic interactions in proteins to the role of biophysics in university teaching courses, were discussed at the meeting. The main virtue of the conference lay in the manner in which a review of a huge number of topics, via sessions centred around either a biological problem or a physical technique, was arranged. This was particularly valuable for highlighting old problems which might now be solved using new methods. Individual sessions did not necessarily provide a balanced view of the field under discussion, but that is always likely to remain the case at the International Congress. The annual meeting of the US Biophysical Society attracts universal attendance and is established as the forum for comprehensive discussions in a large number of subjects. This is more difficult to achieve in a nomadic meeting in which there is a necessarily random sampling of a much wider population. However, for those biophysicists with time and desire to expand their education, looking for a new problem or interested in new methods, the International congresses remain a valuable venue. □

Maxine Clarke is an assistant editor of *Nature*.

Geology

Submersible study of the East Pacific Rise

from members of the *Geocypris* expedition

ON 5 March 1984 the French research vessel *Nadir* docked at Manzanillo, Mexico, at the end of a highly successful leg of diving operations on the East Pacific Rise using the submersible *Cyana*. Several key sites between 11° and 13°N were explored, with the specific aim of studying hydrothermal processes.

Previous investigations by French vessels had established, near 12°50'N, the existence of a narrow zone (less than 200 m wide and 20 km long) of intense hydrothermal activity believed to be due to the presence of a shallow magma reservoir beneath the axis. The East Pacific Rise in this vicinity is conspicuously asymmetric, with off-axis seamounts on one side and linear normal faults on the other. Geological reconnaissances were carried out on four seamounts located 4 km west of the rise axis near 11°30'N(A), 18 km west of the axis at 12°38'N(B), 6 km east of the axis at 12°43'N(C) and 6 km east of the axis at 12°50'N(D).

Seamounts A and C, which are structurally similar and rise less than 400 m above the surrounding sea floor at 2,700 m, were explored extensively. Linear north-south aprons some 500 m long and less than 100 m in relief extend south from the eastern and western margins of both seamounts. Extensive hydrothermal deposits found on seamount C during a *Cyana* dive in 1982. On this leg, highly-altered fragile hydrothermal edifices several metres high were found around an apron of seamount

A. Its western flank consisted of a vertical fault scarp with extensive piles of talus at its foot composed of sulphide boulders and fragments of massive flows. Extensive areas of Fe oxides, hydroxides and thick (> 2 cm) Fe-Mn crusts were encountered during dives from the southern foot of the seamount to its top.

Three dives confirmed the existence of a massive sulphide field capped with ochreous oxidation products on the western limb of an apron of seamount C. Hydrothermal material extends at least 800 m down the south flank of the seamount from its top and some 500 m in an east-west direction along the 2,600 m isobath. Assuming the deposit to be, on average, 3 m thick and to have a density of 3.0 g cm⁻³, its mass is conservatively estimated to be more than 2 × 10⁶ tonnes. Metal contents in the deposit are in the range 20–40 per cent Fe, 1–3 per cent Cu and less than 1 per cent Co. This deposit differs from those on the adjacent Rise axis in its massive appearance, its relative depletion in zinc sulphide and its abundance of hydrated silica. The smaller size (<50 m diameter) and richer metal (Cu and Zn) content of the Rise axis deposits suggest a faster deposition at a higher temperature than occurred for the seamount deposit. The age of the deposit on seamount C is unknown, although the lack of sediment cover indicates a relatively recent origin. The crust on which the seamount lies is approximately 100,000 years old. The occurrence of fresh pillow flows partially covering some of the ochreous hydrothermal products indicates off-axis volcanic activity. Interestingly, near the base of the seamounts at 2,700 m depth the pillow flows are almost entirely covered by pelagic sediment.

A semicircular volcanic structure located between seamount C and the rise axis showed evidence of recent off-axis hydrothermal activity. A diffuse white fluid was exiting from hydrothermal edifices on top of a volcanic peak (<50 m in height) made up of fresh sheet flows, and a large amount of ochreous sediment with slumped boulders of massive sulphide was observed at its base.

Two dives took place on the eastern margin of the axial graben near 12°50'N in order to study the alteration and mode of emplacement of sulphide precipitates at recently active hydrothermal sites. A field of green-coloured altered pillows and flows mixed with sulphide boulders, which formed piles of talus at the foot of a 30 m high fault scarp, was observed. Along the wall was an extensive stockwork of sul-

phide filled veins (<50 m wide) within altered country rock containing chlorite-smectite-sulphide assemblages typical of low-grade metamorphism.

The offset spreading centre discovered by Macdonald and Fox (*Nature* 301, 55; 1983) at 12°53'N was the target for another dive. The eastern segment seemed relatively old, with flows and pillows partially covered by sediment. The western ridge is marked by a small graben (some 10 m deep and 30 m wide) containing fresh lobated flows but discontinuous along the axis. The sporadic occurrence of *Galathea* and brachyuran crabs indicated hydrothermal activity nearby. Proceeding northwards along the western axis the basaltic flows become fresher in appearance (very shiny glassy surfaces) and ochreous sediments more common, suggesting increasing hydrothermal activity. By contrast, the eastern ridge at the same latitude is apparently inactive and being tectonically disrupted.

The first electrical resistivity measurements of the seabed to be made from a submersible were carried out by T.J.G. Francis of the Institute of Oceanographic Sciences, UK. At each site an electrical cable 50 m long was laid along the bottom. Current was passed between electrodes 30 m apart by means of a 12 V battery within the submersible and voltages were detected by Ag-AgCl potential electrodes. The resistivity of the pillow lava terrain was about 40 times that of seawater, in good agreement with downhole logging measurements in DSDP drill holes. The resistivity of the sulphide deposit was between one and two orders of magnitude less than that of the pillow basalts. At one site the seabed was approximately twice as conductive as the seawater and preliminary measurements suggest the sulphides could be of the order of 15 m thick. Self potentials were observed associated with the sulphide deposit.

Electrical potentials were also measured in the water column in order to determine whether hydrothermal fluids exiting from the seabed create masses of water with a different (redox) potential from the adjacent water mass. This experiment, devised by F. Avedik of CNEXO-COB, consisted of measuring the potential difference between one non-polarizing electrode held at 150 m depth and another lowered to the ocean floor at a depth of 2,600–2,900 m. Negative anomalies with amplitudes of 6–8 mV were observed next to the sea floor, some of which extended up to 500–600 m above the bottom. This type of measurement could prove to be a useful way of detecting the presence of hydrothermal activity. □

Contributors to this article were R. Hekinian, F. Avedik, D. Bideau and Y. Fouquet from the Centre Océanologique de Bretagne, Brest, France; T.J.G. Francis of the Institute of Oceanographic Sciences, Wormley, Surrey, UK; J.M. Franklin of the Geological Survey of Canada, Ottawa, and W.D. Nesteroff of the Université Pierre et Marie Curie, Paris.

100 years ago

THE PRIME MERIDIAN CONFERENCE

THE Prime Meridian Conference at Washington on Monday adopted the Greenwich line as the universal prime meridian. Only one vote — that of St. Domingo — was given against its adoption; but the representatives of France and Brazil declined to vote.

To the American resolution for adopting the Greenwich line, Mr. Fleming (Canada) moved an amendment to the effect that the Conference should adopt the 180th degree of longitude east from Greenwich as the prime meridian; but the other British delegates opposing the proposition, it was lost. M. Lefavre (France) said the Greenwich was not a scientific meridian, and that it implied no progress in any science, but was merely a commercial standard. Since, therefore, nothing would be gained to science by adopting Greenwich, France could not make a sacrifice of her own meridian, and incur the vast expense consequent upon the adoption of a new one, because she would thereby gain no advantage whatever. Sir William Thomson said that it was purely a matter of convenience, and that Greenwich answered the world's convenience better than any other standard meridian.

From *Nature* 30, 603, 16 October 1884.

Space programmes

Details of Soviet cosmodrome from space shuttle photographs

from Grant H. Thomson

EVIDENCE that the Soviet Baikonur Cosmodrome is situated at 63.3°E, 46.0°N has already been provided by the remote-sensing Landsat multispectral and return-beam vidicon systems. But that evidence has recently been supplemented by two colour photographs (NASA 84-HC20 and 84-HC-22) obtained using a hand-held camera, with 100 mm and 250 mm lenses, during the flight of STS-9, *Columbia* (1983-116A), in December 1983. Although the ground-resolution of the photography is not particularly good, it is sufficient to add some useful up-to-date detail to maps published by observers of the Soviet space programme.

So far as is known, the Soviet authorities have not yet published, for general reference, a map of the Tyuratam area showing the cosmodrome. They have, however, announced the geographical coordinates of the manned-spaceflight launch pads at 65.5°E, 47.3°N, and press briefings for the Apollo-Soyuz Test Project have the launch site as 66.0°E, 47.8°N, presumably based on Soviet information. These coordinates place the Baikonur Cosmodrome well to the north-east of the extensive launch facilities just north of the old town of Tyuratam. Recently, former CIA official and photo-interpretation expert, Dino Brugioni, has explained how U-2 photography enabled the site to be located, following intelligence reports in the 1950s of a missile site somewhere south of the Aral Sea (*Air Force Mag.*, 108; March 1984). In the absence of any Soviet acknowledgement at the time of such a facility, a war-time German map, showing Tyuratam as a nearby railway station, provided the name by which the cosmodrome has become widely known in the West.

The STS-9 photographs indicate extensive disturbance of the steppe over an area in excess of 150 km², in region C of the accompanying map, which is based on photography. Most noticeable in this area are the three very large new buildings (A) which, because of their size, are probably vehicle assembly buildings. The approximate dimensions of one of these buildings is 220 m × 190 m × 23 m; another is 220 m × 80 m × 35 m; the third has dimensions 155 m × 110 m × 23 m. At B, approximately 2 km to the north-east of these three buildings, the shadow of what is possibly the new heavy-lift vehicle, once referred to as Type G, can be seen. From the solar elevation and the length of the shadow, the vehicle would appear to be more than 90 m tall, in good agreement with Vick's speculation (*Spaceflight* 25, 159; 1984).

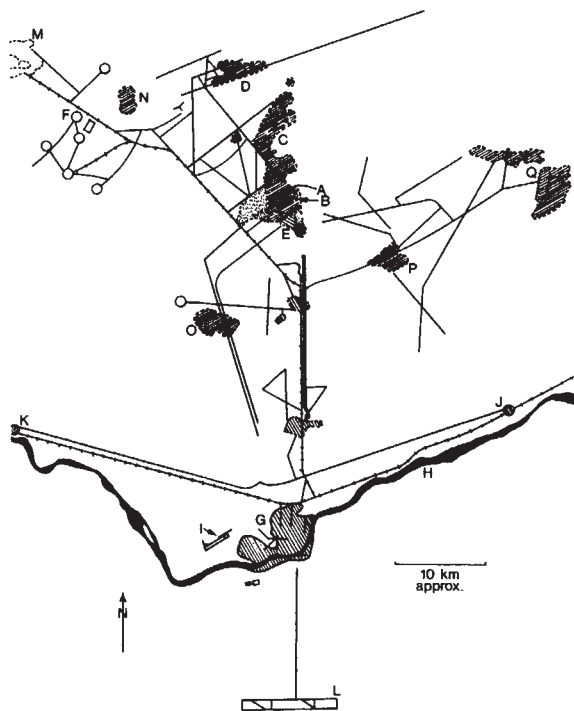
There is a V-shaped feature in the northern part of area C, strongly suggestive of a large new launch pad and associated flame-pit. Its size and probable recent construction suggest that it could be the launch facility for the new heavy-lift vehicle. Area E is shown on Vick's maps as facilities for the long-serving A-vehicle (*The Illustrated Encyclopedia of Space Technology*, 39, Salamander, 1981; and *Soviet Space Programs: 1976-80*, Pt 1, 48, US Govt Printing Office, Washington DC, 1982).

A new runway is under construction at D, in the more northerly part of the cosmodrome. At the time the photographs were taken, this was some 5 km long and

region. This could have been a quarry site and a rectangular structure, approximately 250 m long, can be seen at the north-west limit of the area.

Area F has been identified by Vick as a military launch complex (*Soviet Space Programs: 1976-80*), and possible sites of launch pads or silos are marked with circles. An unidentified rectangular feature, 1,400 m × 750 m, can be seen close to the south-west side of the railway. Sites O and Q show extensive activity, with Q thought to include launch-pad facilities at the end of the railway line. The nature of the large area immediately to the south-east is uncertain, although extensive linear features can be seen. At least two sizeable structures can be seen at P which, although not fully recognizable, is likely to be a technical site.

Just less than 20 km south of Tyuratam are what appear to be parallel runways. If this interpretation is correct, they are likely to be part of an air defence system which almost certainly will have been provided



Map of the Soviet Baikonur Cosmodrome at Tyuratam constructed from photographs taken from the space shuttle *Columbia*.

may not yet have been completed. Extending from and in line with the runway, in the direction of the typical launch azimuth, is a track across the steppe, approximately 20 km long, that may indicate future development. Buildings and signs of construction activity in an area on the north side of the runway are thought to be the Soviet 'space-plane'.

The ground in the area N shows some evidence of disturbance compared with the surrounding steppe, possibly indicating some work in progress at that site. The terrain in the area M appears very uneven, unlike the rolling or fairly flat surrounding

for the cosmodrome. The location would provide airspace clear of the launch complex.

Although the photographs do not provide great detail, they clearly show the extensive activity now in progress at the Baikonur Cosmodrome and provide a good guide for the West to the Soviet authorities' intention of continuing with their investment in space programmes. □

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Astrophysics

Neutrinos and the Sun

from Virginia Trimble

NOTHING can replace real data from a working scientific experiment. This was the most important lesson to be learned by the participants in the Homestake Neutrino Conference*. Among the questions addressed, for which there either are, or should be such data, were the following. (1) What is the flux of neutrinos hitting the Earth? (2) How does it depend on time and neutrino energy? And (3) what is the Sun doing in addition to (or instead of) making neutrinos? In short, the answers are, for (1), still puzzlingly low; for (2), possibly variable with an 11 yr period; and for (3), perhaps stirring its interior, probably driving its magnetic field with a dynamo at the base of the convection zone, and, just maybe, showing a solar activity cycle as far back as 700,000,000 yr.

First the incident flux. There cannot be a physicist, astronomer, or chemist who has not heard of Raymond Davis Jr and his 100,000 gallons of cleaning fluid (C_2Cl_4) in the depths of the Homestake Mine. The experiment was designed to permit recovery of a few atoms of ^{37}Ar , resulting from the decay of ^{37}Cl driven by neutrinos from the Sun. The counting rate from the recovered atoms has persisted for more than a decade in being about one-third of what theorists predict from solar neutrinos alone (observed production rate 0.39 ± 0.05 atoms per day = 2.1 ± 0.26 SNU, after subtracting the current best estimates for assorted backgrounds). There is now no serious doubt about the atoms' identity: they not only have the chemistry and decay energy of ^{37}Ar but also have its half life (J. Rowley, Brookhaven National Laboratory). But the fraction of ^{37}Ar production that should be attributed to backgrounds from cosmic-ray secondaries has become less certain. Two backgrounds are important, muons and neutrinos. E. Fireman (Center for Astrophysics) is currently checking the muon background adjacent to Davis's tank using several thousand gallons of potassium hydroxide. The rationale behind his experiment is that cosmic-ray particles can be expected to make ^{37}Ar from ^{39}K at about the same rate as from ^{37}Cl , whereas neutrinos will make far less. His 1 σ limits on particle production of ^{37}Ar are currently 0.20 ± 0.20 atoms per day, consistent either with the standard best estimate of 0.08 atoms per day or with the entire observed rate of 0.47. Longer runs should tighten the error bars considerably. The flux of cosmic ray-produced neutrinos can easily be checked only by $e-\nu$ scattering at energies ≥ 10 MeV (because of interference from still other kinds of backgrounds at lower energies) using

multi-tone water Čerenkov detectors. At least five of these are now operating and producing consistent results (T. Stanov, Bartol Research Foundation). The detector in Kamioka, Japan, however, has seen a considerable excess of low-energy neutrino-like events. If these result from stopped muons and the same flux hits the perchlorethylene tank, it will produce $0.37^{37}Ar$ per day, essentially the observed level.

An estimate of neutrino flux as a function of energy is vital for deciding whether the low flux at Homestake is due to a minor flaw in the solar model (affecting only the high-energy B^8 neutrinos), neutrino oscillations during propagation (which might reduce the flux at all energies by about a factor of three), or some major defect in modern astrophysical theory (for example, stars do not derive their energy from fusion and so do not produce any neutrinos at all). Apart from the Davis result, there are currently only upper limits, mostly several orders of magnitude above predicted values and pertaining to fluxes made by high-energy cosmic rays (P. Sokolsky, University of Utah) or stellar collapses (A. Burrows, SUNY) rather than by the Sun.

Contributions on the time-dependence of neutrino flux were rather more intriguing. Rowley's simultaneous plot of the ^{37}Ar production rate and sunspot numbers since 1970, particularly the coincidence of the very high run nos 27 and 71 with the giant flare of 4 August 1972 (another indicator of solar activity) strongly suggested a correlation. A. V. Kopylov (Institute for Nuclear Studies, Moscow) pointed out that the present data are not inconsistent with constant ^{37}Ar production rate, and R. Lingenfelter (University of California, San Diego) sketched out calculations eliminating the most obvious sort of connection: energetic particles from a large solar flare produce neutrino secondaries at a rate too low by 10^6 to affect the Homestake experiment. Nonetheless, the possibility of correlated time-dependence is clearly worthy of further study. It is therefore particularly disturbing that the Brookhaven experiment will have to be wound down over the next fiscal year unless a new source of support is found.

Several promising experiments are under construction. The Institute for Nuclear Studies, Moscow (N.V. Gavrin, A.V. Kopylov) has 25 tonnes of gallium metal on hand, and more available, and a chamber ready and lined with iron and concrete shielding at 3,500 m depth at Baksan. The group hopes for construction in 1985 and counting in 1986. $^{71}Ga(\nu, e)^{71}Ge$ has a low enough threshold to see neutrinos from ordinary pp reactions. The

same group is also working on both a scaled-up ^{37}Cl experiment (five times Davis's volume; the equipment is ready, but the chamber still needs to be dug) and $^7Li(\nu, e)^7Be$ (for which they have tested recovery and counting techniques for a 12-litre tank and are ready to try 200 l). The Cl experiment should provide a high enough count rate to see any solar cycle variations unambiguously, and, with patience, annual ones, while the Li experiment is sensitive to CNO cycle and pep neutrinos, thus testing different details of nuclear and solar physics.

MPI Heidelberg (W. Hampel) intends to continue a gallium experiment, in the form of $GaCl_3$, despite Brookhaven's withdrawal from the collaboration. They have tested germanium recovery and counting in a 1.25 tonne tank and envisage a 30 tonne experiment at an existing European site. L.S. Peak (University of Sydney) reported that his group is about to install its first 10 tonne module of a projected 100 tonne water Čerenkov detector to look for a $\nu-e$ scattering at the Broken Hill mine. In practice, this is sensitive only to B^8 neutrinos, owing to high backgrounds at low energies, but it is otherwise totally different from the Cl experiment and can yield approximate directional information, separating the Sun from other sources.

Models of the solar interior designed to reduce the predicted neutrino flux are numerous and often rather *ad hoc*. G. Michaud (University of Montreal) and E. Schatzman (University of Nice) reviewed the effects of turbulent diffusion. If this provides a diffusion coefficient about 100 times that expected from ordinary molecular diffusion, then fresh hydrogen coming in reduces the necessary central temperature slightly and hence the predicted flux by about the requisite factor of three. Energy to drive the turbulence and mixing can be drawn from the build-up of burnable 3He just outside the solar core (I. Roxburgh, University of London). One price paid is a 30 per cent increase in main-sequence lifetimes for low-mass stars, and thus some difficulty in interpreting the Hertzsprung–Russell diagrams of globular clusters. A partial compensation is a better understanding of lithium abundances on the surface of old stars.

Efforts to probe the central temperature, composition, rotation and magnetic field distribution (all of which can affect neutrino production) of the solar interior using frequencies of g-mode oscillations would seem premature. G. Isaak (University of Birmingham), whose group has collected much of the solar surface velocity data concerned, does not believe that these modes have yet been unambiguously identified. The p-modes have been seen in considerable detail, but are only informative about the solar core after the dominant contributions from the envelope have been understood and subtracted.

Nonetheless, p-modes may already have

*Lead, South Dakota, 23–25 August.

resolved one uncertainty. P. Gilman (High Altitude Observatory) reviewed the discrepancy between solar dynamo models with driving in the convection zone and models for convective driving of the solar differential rotation. The former require angular frequency to increase inwards (to make active zones migrate to lower latitudes through the solar cycle), while the latter require angular frequency to decrease inwards. The observed p -mode-splitting strongly favours ω decreasing inwards (see Duvall, T.L. Jr *et al.* *Nature* **310**, 22; 1984). Gilman suggested an alternative site for dynamo driving at the base of the convection zone, where ω seems to be rising again with depth.

Finally, P. Foukal (Atmospheric and Environmental Research Inc.) showed fragmentary sunspot, auroral and ^{14}C data suggestive of a roughly 11 yr solar activity cycle for several hundred years before the late-seventeenth-century turn-off (the

Maunder minimum). And, in what was perhaps the greatest surprise of the meeting, he showed photographs of a pre-Cambrian varve sequence discovered by George Williams of Broken Hill Proprietary. Varves are layers deposited in periglacial lakes when rapid spring-summer melting and sediment transport alternate with quiet winter settling of silt. The annual layers showed additional banding with periods ranging from 9 to 14 yr and averaging 11.2 yr over a 20,000 yr sequence. This strongly suggests an influence by the solar cycle some 700,000,000 yr ago. There's nothing quite like real data, but you can never be sure where it's going to come from. □

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Invertebrate neurophysiology

Sodium channels in muscle

from P.R. Stanfield

INFORMATION in the nerve fibres of both vertebrates and invertebrates is signalled by means of action potentials that are generated by an inward movement of sodium ions through channels (Na channels) which are selective and dependent upon membrane potential. Similar channels characterize most of the skeletal muscle fibres of vertebrates, where the all-or-nothing action potentials they produce trigger contraction by releasing calcium ions from an intracellular store in the sarcoplasmic reticulum. In contrast, invertebrate muscles have seemed to lack sodium channels, showing instead an electrical excitability that depends on the presence of calcium channels in the muscle fibre membrane and is either graded or all-or-nothing in nature. This calcium-dependent pattern of electrical excitability has been seen as phylogenetically older than the pattern in vertebrate muscle and Ca channels are themselves believed to be evolutionarily older than Na channels¹.

The generalization that invertebrate muscles lack Na channels has recently been challenged by Lawrence Schwartz and Walter Stühmer², who have used electrophysiological techniques to show that the striated muscle fibres of the planktonic arrow worm, *Sagitta elegans*, possess Na channels and certain other properties that make them very like skeletal muscle fibres of vertebrates. *Sagitta* was chosen because it belongs to the phylum Chaetognatha and in turn to the deuterostome branch of coelomate animals, the branch to which the vertebrates also belong.

In discussing their data, Schwartz and Stühmer argue that, since Na action potentials are always more rapid events

than those depending on Ca^{2+} , they permit a more rapid switching on and off of muscle activation. The advantages of a rapid switch may have provided part of the selection pressure that led to the expression of Na-channels in muscle as well as in nerve. The rapid muscular responses required of a successful predator like *Sagitta* may account for its adoption of Na channels. The alternative, however, is that they are the bequest of an evolutionary ancestry shared with the chordates, and that the Na action potential in muscle is a deuterostome trait.

If this is a deuterostome trait, it is not a universal property among the group, nor is it limited to them. Indeed, since Na channels of very consistent properties are almost universal in nerve, it is possible that their expression in muscle has arisen independently in a number of groups. Thus, the tunicates (urochordates) lack muscle Na channels, while *Amphioxus* (among the cephalochordates) possesses them; these protochordates have therefore been seen as transitional between non-chordates that lack muscle Na channels and the vertebrates¹. Furthermore, among the vertebrates, lack of Na channels lives on in smooth muscle and also in the slowly-contracting tonic skeletal muscle fibres, found in frog and, for example, in the extraocular muscles of certain mammals. Moreover, protostomes (the other branch of the coelomates) can also have muscle Na channels, for these have now been demonstrated in an evolutionary ancient group of arthropods, the scorpions³.

While muscle fibres of the scorpion and of *Sagitta* both give brisk twitch-like contractions in response to Na-dependent

action potentials, they are very different in other ways. First, in scorpion, the Na action potential is followed by a brief train of Ca-dependent ones³, an electrical behaviour that has not been described in other muscles. Second, blockage of Ca channels or removal of external Ca^{2+} blocks contraction in the scorpion, in spite of the presence of a well-developed cellular store of Ca^{2+} . This dependence on external Ca^{2+} is commonly found in invertebrate muscles that lack Na channels. In *Sagitta*, as in vertebrate muscle, removal of external Ca^{2+} seems not to block contraction².

The presence of Na-channels in scorpion muscle may raise the question of whether Ca-dependent excitability really is the older form. There remains little doubt that it is, and arguments from ontogeny support the position. During development, vertebrate muscles go through a stage where they possess only Ca action potentials. And even in adult muscle, Ca channels are retained at a high density. In addition, in the tunicates, whose muscles lack Na channels, these channels are lacking in all cells in the initial stages of development (though they are present in the unfertilized egg) and are never expressed in presumptive muscle cells. Indeed, these cells acquire their mature pattern of ionic permeability very early in embryonic life, and at much the same stage at which Na channels first appear in presumptive nerve cells⁴.

To confuse the picture, certain muscle fibres seem to have the potential but not usually the stimulus to express Na channels. Thus denervation of tonic muscle fibres of the frog leads to the development of Na action potentials; since reinnervation suppresses them again, some trophic effect of nerve seems to be involved⁵. Denervation of crayfish muscle has similar results⁶.

At the end of their article, Schwartz and Stühmer challenge comparative physiologists to use new methods to investigate muscle cells hitherto inaccessible to electrophysiological attack. Since it was in a crustacean muscle that the first evidence for the Ca channel was obtained⁷, since the pattern of evolution of muscle excitability turns out to be complex, and since a comparative approach will help sort out the poorly understood problem of excitation-contraction coupling³, there is a strong case for a wide survey. □

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The nature of tumour cell proliferation

SIR — The comments by Erika Coward¹ on the origins of malignancy were interesting though not entirely novel. Clearly, cancer is not just a matter of abnormal proliferation and it would seem necessary to invoke lesions which do more than alter the levels of regulators otherwise one would merely have hyperplasia. Nevertheless, it is equally evident that the major, and possibly only, defect which is common to all transformed cells is that they replicate when they should not do so. Such an effect can be induced in a variety of ways, each of which could produce a unique functional change, while operating through the same mechanism². The ability to totally inhibit proliferation would be a major advance but it might not overcome the effects of the altered properties, for example, increased hormone production.

Bell³, has considered some aspects of altered proliferation consequent upon changes in the levels of regulators, both inhibitory and nitrogenic. On the other hand, as a result of my deliberations on the nature of proliferation processes^{2,4}, I have suggested^{5,6} that the aberrant behaviour of cancer cells in most cases is due to an altered threshold with respect to regulator levels — decreased with respect to mitogens, raised for inhibitors. (A threshold is required by the cell cycle concept I have developed^{2,5}, although it may vary in magnitude and can be zero in particular instances. Its existence provides an explanation for cell density inhibition of proliferation while an altered threshold accounts for the often observed effect thereon of the malignant transformation, namely the ability of such cells to attain much higher densities⁵.) At the same time, I also briefly considered the effects of altered levels of regulators on surrounding normal tissue cells⁵.

There is no evidence that tumour cells in general proliferate faster than the corresponding normal cells. However, although taking different approaches, both Bell and I concluded that a critical tumour size might exist above which the growth may increase more rapidly than hitherto (one possible explanation for latency). My studies⁵ also indicated the possibility that tumours of limited growth potential might arise as a result of lesions which increase the uptake⁷ or binding of regulators, whereas those affecting thresholds could (depending on the magnitude of the change) yield tumours more malignant with respect to growth potential.

A question of major importance to Coward's considerations, however, is whether or not regulation *in vivo* is ever effected by a single agent. Culture studies clearly indicate that the replicative ability of cells can be affected by a wide range of normal agents, including various hormones. Recent studies have emphasized that transformation is often

associated with positive growth factors (mitogens) rather than inhibitors. I have proposed a mechanism for the interaction of regulators² and, on that basis, suggested⁶ (in contrast to the chalane approach) that attempts be made to control the proliferation of tumour cells by the use of a combination of such agents rather than just one (thereby limiting side effects on the replication of normal cells). However, the available data fail to indicate which regulators should be used, and at what concentrations, in any particular instance. It would thus still seem necessary to establish suitable regimes by trial and error, as is the usual procedure.

Another problem is that of heterogeneity in the cells of a tumour. Such heterogeneity implies that the response to growth regulators will also be heterogeneous⁵. As a consequence, treatment by the methods proposed by Coward and by myself, are likely to meet the same limitations in this direction as present approaches. On the other hand, they may be less toxic for the patient and might not produce the opposing adaptive responses.

Interestingly, accompanying Coward's letter was another by Schleifer *et al.*⁸ on the effects of psychological factors on the immune response. That brings to mind the old issue of whether or not the mental state can affect tumour formation and development, a topic about which there has been much (often subjective) discussion. There is much data showing that various hormones can regulate cell proliferation, both negatively and positively. It thus seems reasonable to accept that any process modulating hormonal levels, such as stress, is potentially able to modify tumour growth rate (if not transformation), although the actual dependencies are likely to be complex and individualistic.

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Machine-readable DNA sequences

SIR — Hayashi and Munakata¹ suggest that the four bases in DNA might be represented by four musical notes. We prefer a graphic representation, where the four bases are displayed in a line extension format: values attributed to the four bases G, A, T and C being +2, +1, -1 and -2. In the example (below), 100 nucleotides of coding sequence from a recent paper² have been converted into this format: significant features of the sequence ("TATA" transcription signal — in fact TATTAT here; start site of transcription; translation initiation codon ATG) are indicated.

Such a presentation has several interesting features. First, particular sequence patterns are readily discernible: purine (A, G) rich stretches appear exclusively above the line and pyrimidine (C, T) stretches below. G/C and A/T rich regions have extended and retracted aspects respectively. Second, restriction sites appear (usually) as simple

rotationally-symmetrical patterns which are rapidly picked out by the eye. Third, it takes little time to determine, for instance, that the dinucleotide pair C followed by G is, as expected, depleted. Fourth, to convert to the complementary strand the diagram need only be rotated through the horizontal axis.

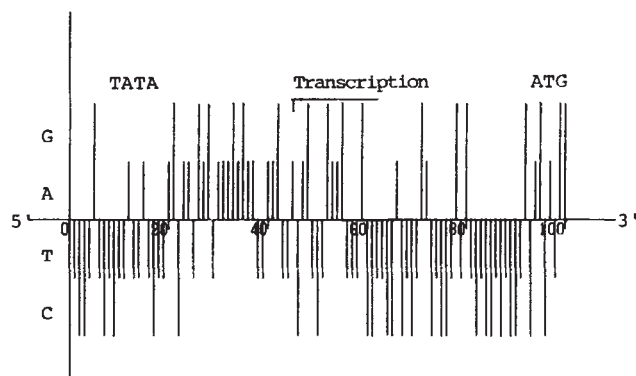
Such a line extension format is inherently machine-readable, and may permit suitably equipped readers to transfer sequences directly from the printed page to an information retrieval system.

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A sequence of 100 nucleotides from a mouse major histocompatibility complex gene, *E_β*

Organizing centres for three-dimensional chemical waves

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Excitable media propagate periodic waves of chemical activity. In three dimensions, they resemble scrolls rotating about axes where reactions are not periodic. These axes close in rings which may be linked and knotted, in ways limited by an exclusion principle.

NONLINEAR waves are conspicuous in a variety of biologically and chemically excitable media. Waves of excitation propagate at uniform speed and mutually annihilate in collisions in the Belousov-Zhabotinsky reagent¹⁻⁸, in retinal⁹ and cortical¹⁰⁻¹² nerve nets, where they may underlie neuromotor pathologies such as epilepsy, in heart muscle¹³⁻¹⁶ where they are involved in life-threatening arrhythmias, and in aggregations of social amoebae¹⁷⁻²⁰ where they coordinate normal development. These waves commonly appear as involute spirals radiating from tiny rotating activity patterns, here called 'organizing centres'. In three-dimensional media, spirals are still seen, now as cross-sections through a scroll-shaped wave^{3-5,21-28}. The scroll emanates from its central organizing axis, which typically forms a closed ring. The simplest form of organizing centre is an 'untwisted scroll ring'^{11-4,21-28} or 'toroidal vortex'²⁹. We present here a chemical/topological exclusion principle that predicts a quantized hierarchy of other, topologically distinct organizing centres and delimits the initial conditions required for their experimental realization²²⁻²⁸. We now review the published theoretical, computational, and experimental evidence concerning these three-dimensional reaction-diffusion structures.

Scroll rings

Periodicity is the central principle of all these arrangements of waves. Throughout any region organized by a spiral wave (Fig. 1), each point cycles periodically through activity, refractoriness, quiescence, and activity again. Hence we may describe a snapshot of the pattern in terms of the spatial distribution of phase. The phase, ϕ , of a point means the fraction of a cycle elapsed since the last wavefront excited that point. We represent the phase geometrically as a point on an abstract cycle, the unit circle S^1 .

Description of spiral patterns in terms of phase entails a weird topological consequence: there must be a 'phase singularity' in the medium^{1-3,23,30}. To prove this, first consider any closed path C enclosing the spiral's inner portions (Fig. 2). In one circuit of C , phase changes through exactly one full cycle. In mathematical terms^{1,31}, the map $\phi|_C: C \rightarrow S^1$ has winding number $W = \pm 1$. To conclude the proof, a theorem of topology³¹ asserts that since $W \neq 0$, phase cannot be assigned continuously throughout any disk bounded by C . The most localized discontinuity possible is a phaseless point surrounded by all phases of the cycle: a phase singularity. The immediate neighbourhood of the singularity is a rotating pattern of chemical activities, the pivot of the rotating spiral wave, and the centre from which it radiates.

Because even a thin layer of excitable medium has finite thickness, the ostensibly flat spiral is actually a cross-section of a three-dimensional wave shaped like a scroll. This wave emerges from a filament of singularity. The filament has no physical substance; it cannot be strained out of the liquid like a strand

of spaghetti. It represents merely the locus of intersection between two surfaces of uniform chemical concentration^{1,3,21-23}. As such, it typically closes in a ring (unless interrupted by the physical boundaries of the medium as in Fig. 2). This predicted exact closure was confirmed experimentally by reconstruction of the fixed wave from serial sections^{28,32} and by recent direct video recording during the uninterrupted reaction⁴, as well as by computer simulation from the equations of local kinetics and molecular diffusion^{3,29}. In the Belousov-Zhabotinsky reagent, these scroll rings persist through about a hundred rotations of the scroll, shrinking slowly at a rate which increases with the curvature of the singular filament^{4,21,29,32}.

A symmetric caricature of the observed scroll ring is shown in Fig. 3a, which depicts a snapshot of the expanding wavefront of excitation. In the next instant, the front would advance a uniform distance at every point and the ring would shrink slightly.

Twists and links

New topologically distinct possibilities exist for scroll ring closure. Imagine forming the ring by bending a straight segment of scroll, then sealing its ends together to preserve continuity (Fig. 3b). We might instead have given the straight scroll a 360° twist (Fig. 3c), or even have tied knots in it before joining its ends. These structures are indistinguishable locally: in each, a short segment along the singular filament is indistinguishable from a segment of the scroll ring of Fig. 3a. Because that untwisted, unknotted scroll ring is viable, local considerations suggest that twisted and knotted rings might be comparably stable.

However, global topology forbids most of these otherwise-plausible organizing centres. Consider, for example, the once-twisted scroll ring (built on the pattern of Fig. 3c) that has evolved to fill a cube of the medium. The winding number argument used above to detect a singularity reveals that this solitary once-twisted ring cannot exist: it must be threaded by another singular filament. To prove this, we evaluate the winding number along the inner equator of the invisible torus that confines Fig. 3b and c. As shown in Fig. 4, W is the number of twists locked into the planar scroll ring. Because $W \neq 0$, any surface bounded by that equator must be punctured by a singular filament^{1,24,31}.

In a computer-generated image^{24,27,28} of the fully evolved wave (Fig. 3d), spirals emanate from the singular ring and collide awkwardly along a gnarled helicoidal surface, whose axis is the topologically-inferred phase singularity. This image was constructed by placing an involute spiral in each half-plane extending radially from the ring's axle, and then twisting it through 360° while swinging the plane once about the axle. Gomatam⁵ has also shown analytically that such 'screw

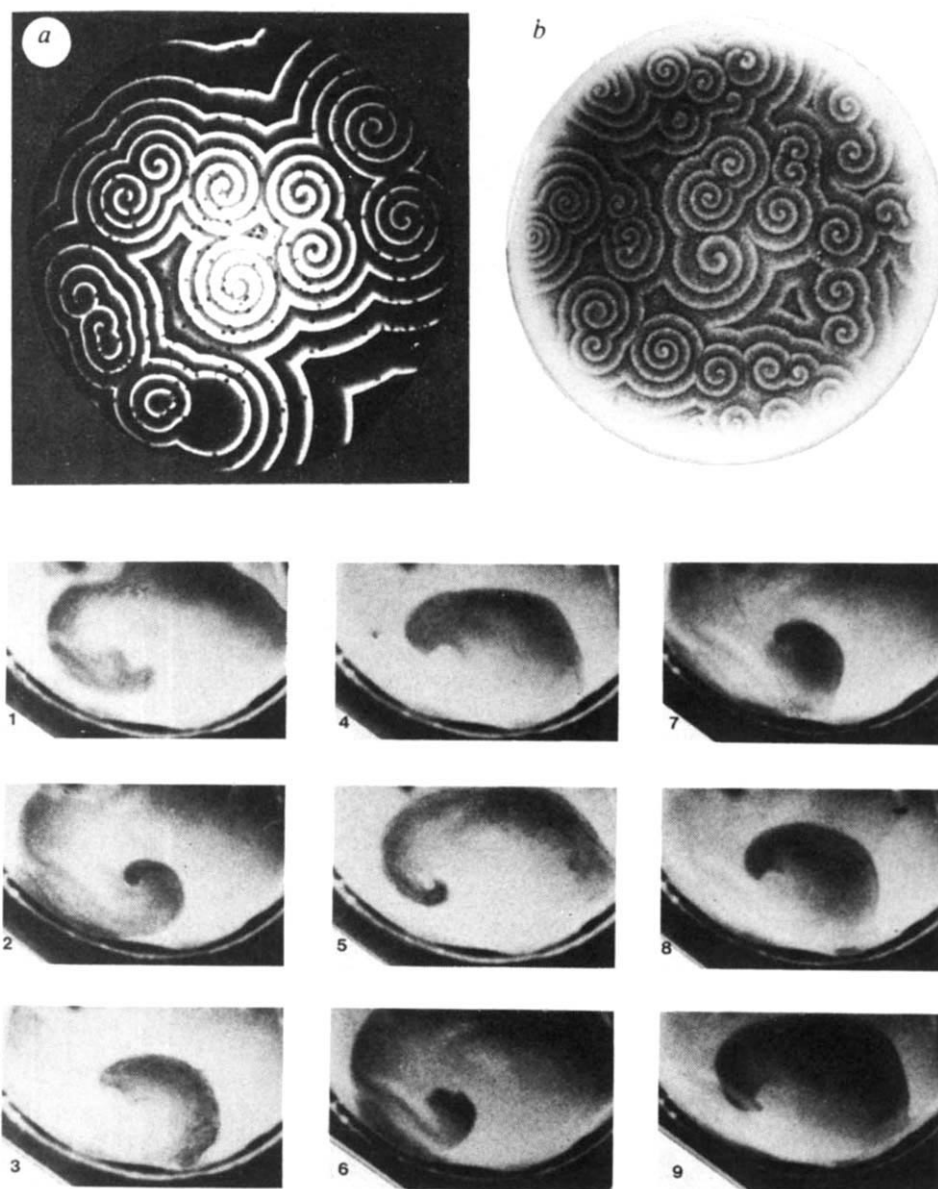


Fig. 1 Rotating spiral waves in excitable media. *a*, Transmitted light photograph of the chemical reagent originated by Belousov⁴², and modified^{2,7,21}. Waves radiate from spiral centres at a few mm min⁻¹, the spirals turning in about 1 min. The liquid layer is 1 mm deep; the field of view spans 90 mm. *b*, Dark-field photograph of a monolayer of *Dictyostelium discoideum* cells. Cells refract light differently when responding to a pulse of cyclic adenosine monophosphate. The instantaneous loci of this response are spirals rotating once in ~5 min as waves propagate at a few mm min⁻¹. The field of view spans 90 mm (with permission from Newell²⁰). *c*, Successive photographs of the retina of a chicken taken by ambient light at intervals of 40 s. The dark area consists of cells recovering behind a wavefront of spreading depression. The front moves ~3 mm min⁻¹, rotating once in 3 min (twice in this mosaic). The field of view is 11 mm × 7.5 mm. (Adapted with permission from Gorelova and Bures⁹).

surface is a solution to linearized reaction-diffusion equations. Because physical rotation is equivalent to time evolution for this particular solution²⁴, we can animate the intricately rotating, propagating, and self-colliding wavefront by merely spinning it about its vertical axle (ref. 28, and our videotape).

However, there are two reasons why this symmetric solution is best regarded as a limiting extreme of a physically more realistic case. First, observation shows that stable spiral waves in excitable media always radiate outwards from their singularity; here they have been allowed to converge to the axial singularity in Fig. 3*d*. Second, to avoid ending the wave along an unphysical edge, the symmetries of Fig. 3*d* require it to extend to infinity in all directions, in violation of the realistic requirement that waves propagate at finite speed.

The practically realizable (but analytically less convenient) alternative is a pair of identical linked scroll rings. Each provides the necessary threading singularity for the other. Figure 5*a* represents this organizing centre with the horizontal ring viewed nearly edge-on, while the other, regarded as arbitrarily large, is vertical, but hidden behind an interface where waves from each source collide. In Fig. 5*a*, viewports are opened in the horizontal scroll wave as it approaches collision a finite distance from the much larger vertical source ring. Realism is improved in Fig. 5*b* by giving both rings identical radii. Figure

5*b* presents a schematic of comparably sized rings, interlinked by their mutual rotation.

This mated pair of linked scroll rings exists in two mirror-image isomers, each with both singularities left-twisted (as here) or right-twisted. Either can be constructed from toroidal surfaces of uniform chemical concentration^{23,24}. Experimental methods to contrive such initial conditions in an excitable medium are suggested elsewhere^{23,24}. If its laboratory synthesis is successful the pair of linked twists will be the second observed organizing centre.

Exclusion principle

The allowed anatomy of an organizing centre is quantized by an exclusion principle, derived from both chemical and topological requirements. Away from boundaries of the medium, surfaces of uniform concentration must be disjoint, closed, and orientable; infinite concentration gradients are forbidden; singular filaments must be wave sources, not sinks; and far from the organizing centre, concentrations must approach quiescent uniformity²³. The exclusion principle then states²⁶ that any realizable organizing centre must satisfy the constraints that:

$$\sum_{j=1}^R L_{ij} N_j = 0 \text{ for each } i = 1, 2, \dots, R \quad (1)$$

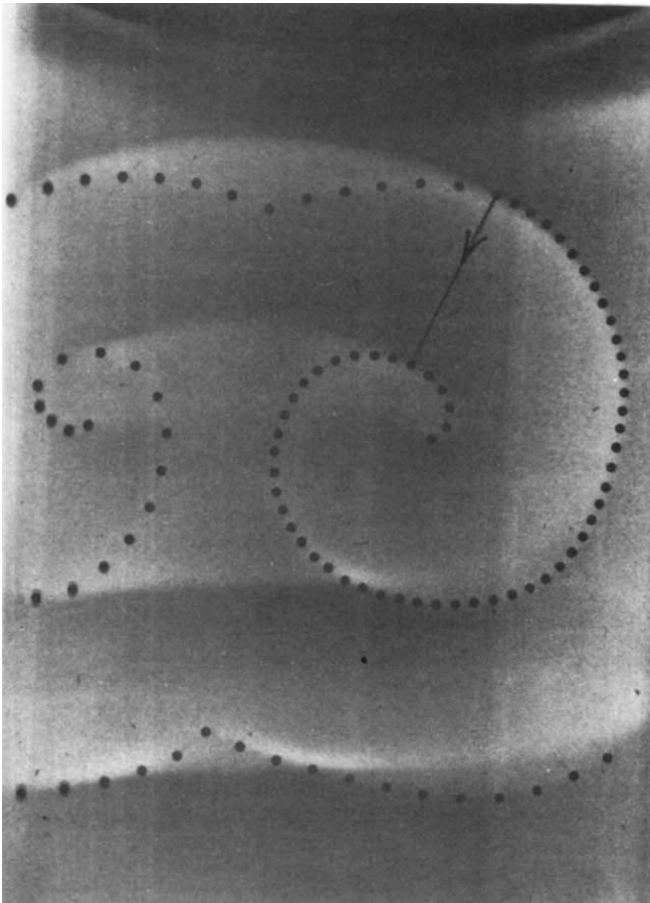


Fig. 2 Untwisted scroll wave and singular filament. A scroll wave in modified⁴ Belousov-Zhabotinsky reagent is outlined in dots added to the photograph. Its spiral cross-section is exposed where it encounters the glass wall. A closed curve is traced 360° along this spiral with no change of phase (the chain of closely-spaced dots) then radially through one full wave (the line segment) passing through one full cycle of phase. It is accordingly linked by a singular filament: the scroll's source. (Reproduced with permission from Welsh *et al.*⁴.)

where R is the number of constituent rings or knots²⁵, N_j is the positive number of spiral arms emanating from ring j (the singularity's "topological charge"¹⁶), L_{ij} is the linking number of rings i and j , and L_{ii} is the linking number of ring i with an imaginary ring traced along the wavefront infinitesimally nearby. These equations dictate the unique integer number L_{ii} of revolutions of the spiral wave required along each ring: $N_i L_{ii}$ must be equal and opposite to the sum of that ring's signed linkages with each of the other rings, counted with multiplicities N_j . L_{ii} is a sum of two real numbers called^{25,33,34} the 'twist' (an integral of the spiral's gyration around the ring) and the 'writhing' (which depends only on the shape of the filament itself, and is zero for planar rings).

For a solitary ($R = 1$) ring or knot, the principle requires $L_{ii} = 0$. Thus, it predicts that a solitary scroll ring cannot contain net twist, as proved earlier by winding number arguments, and a solitary scroll knot must be twisted by an amount equal and opposite to its writhing number^{25,26,34}. An untwisted ring pair cannot link. A pair of rings may contain equal twists if appropriately linked. For organizing centres composed of more than one ring, equation (1) specifies the set of quantum numbers, N_j , L_{ij} , and L_{ii} compatible with the known constraints. There may be additional constraints forbidding some organizing centres allowed by equation (1).

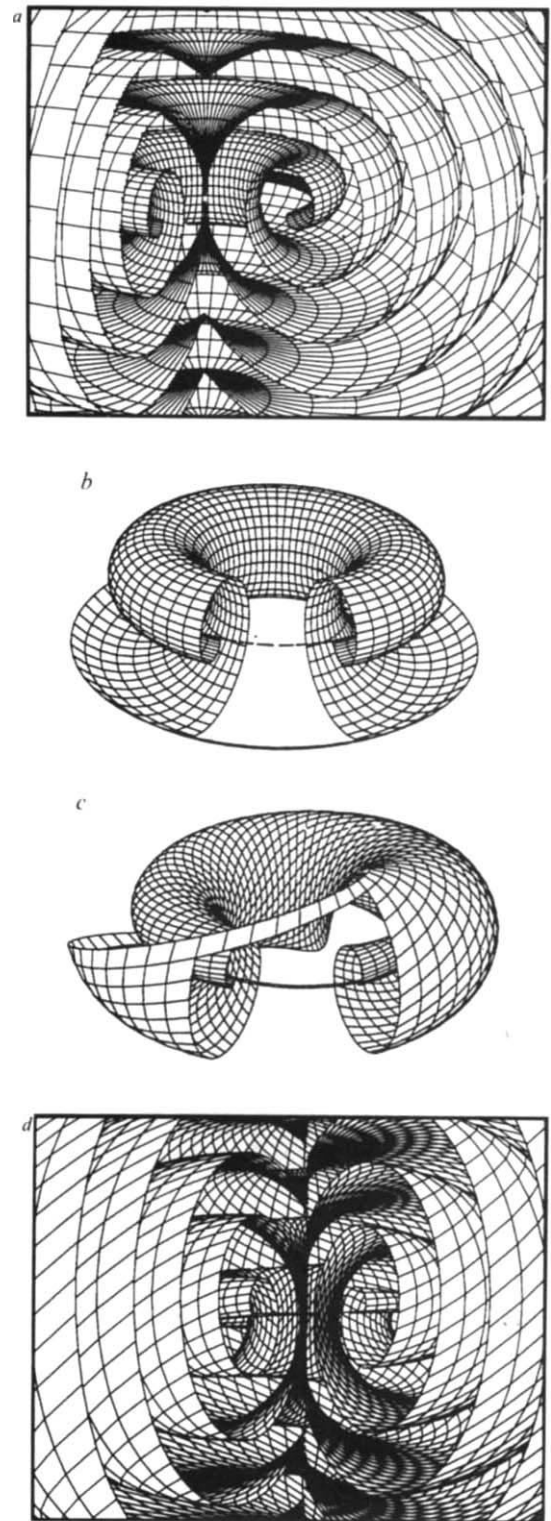


Fig. 3 Computer images of untwisted and twisted scroll rings. *a*, The inner lamina of an untwisted scroll ring wave fill the field of view. Their source is a horizontal circle of phase singularity, the front's inner edge. To aid in visualization, wavefronts are made transparent within a wide sector reaching to the internal symmetry axis along which wavefronts collide. (Reproduced from ref. 28.) *b*, At the same scale, we show as much of *a* as fits inside a torus around the singular ring. The wave's edge is a closed ring. *c*, As in *b* but the contents of the invisible torus are given one full 360° left twist around the circumference. The outer edge of this wave (where it terminates on striking the torus) is still a closed ring, but it now links the torus. *d*, As in *a*, but the wave field is extrapolated from *c* rather than from *b* to fill space. A phase singularity linking the torus is unavoidable. Here it is depicted as the unphysical focus of converging waves. (Reproduced from ref. 28.)

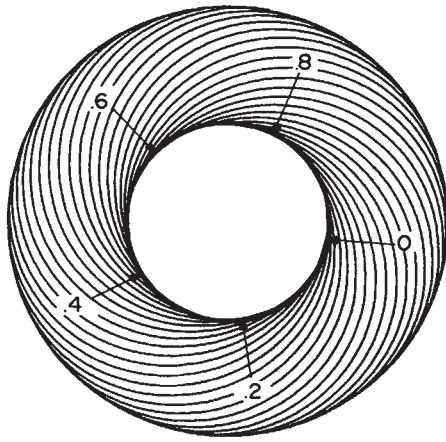


Fig. 4 Successive wavefront positions on a twisted scroll ring. Closed contours of fixed phase on an imaginary toroidal shell enclosing a uniformly once-twisted scroll ring. Only half of each contour is visible. A disk plugging the hole in the toroid touches these phase contours in serial order, spanning one cycle around the disk's boundary. Since $W \neq 0$, the disk must contain a phase singularity³¹. Applying the same argument to every diaphragm bounded by the toroid's inner equator, we detect an unforeseen singular filament threading the twisted scroll ring^{1,24}.

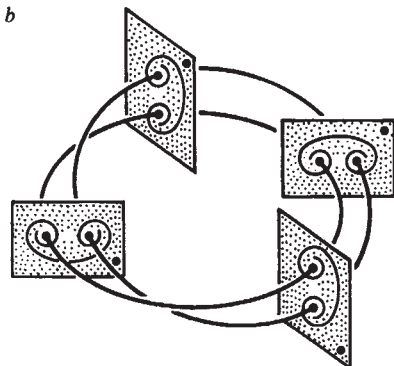
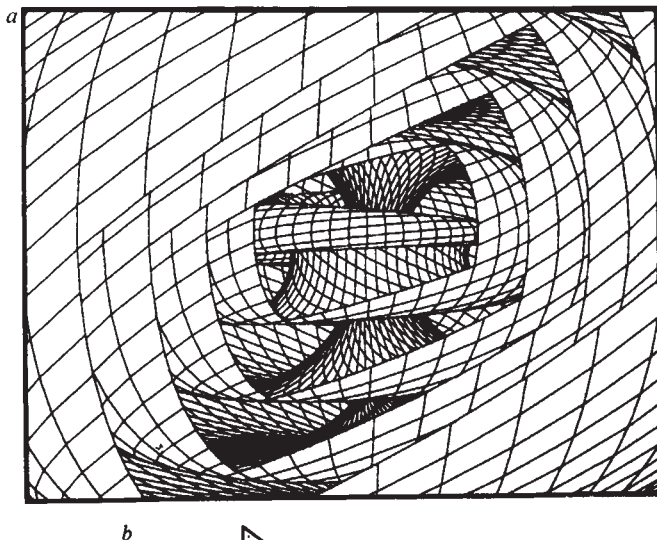


Fig. 5 Mutually linked pair of left-twisted scroll rings. *a*, As in Fig. 3*d*, a once-twisted scroll wave radiates from a horizontal source ring (exposed through a succession of windows). But here the wavefront stops short of collision with itself along an unphysical 'sink'. Rather, it collides with the outgoing wave from the much larger vertical twisted scroll ring hidden in the interior. (Reproduced from ref. 28.) *b*, A mirror-image pair of spirals is transported around a circular path to sweep out a pair of adjacent scrolls. Four local cross-section planes are shown. The pair is rotated as it is transported, so that each scroll is left twisted and the two are mutually linked. Waves emerging from the pair in each cross-section plane eventually join in rings. (Reproduced from ref. 24.)

Transmutation

Solitary scroll rings tend to contract towards their centres of curvature^{4,21,29}; it seems likely that the twisted rings of more complex organizing centres do the same. Unless neighbouring filaments repel one another²², organizing centres in media without boundaries must then eventually decay, leaving behind only quiescent uniformity. (Assuming that there are boundaries, a ring retains the alternative of opening into a scroll wave with an uncurved and stable axis.) Elementary decay processes^{3,21} can only involve fission or fusion of rings. It is possible to envision such events in terms of continuous deformation of concentration fields²⁶. The result in each case is a rearrangement of quantum numbers which alters R by ± 1 and preserves identities (equation (1)). One allowable transmutation pathway regresses from a solitary trefoil knot to a linked pair of twisted scroll rings to a solitary untwisted scroll ring to an unlinked pair of scroll rings or to spatial homogeneity ('vacuum'). The latter two transmutations have already been observed in a chemically-active medium³⁷. Presumably they can also occur in reverse, including the final transmutation to/from the vacuum state. In particular, mechanisms for creating scroll rings *ex nihilo* may have practical importance in biologically excitable media such as heart muscle³⁸.

Conclusion

We envisage a hierarchy of organizing centres ordered by the number of constituent rings. Loosely analogous to the Periodic Table, this hierarchy contains atoms made of scroll rings, much as in the 'vortex atom' notion of Thomson and Helmholtz^{39,40}. At each level, diverse isotopes are distinguished by mutual linkage and knotting of rings. Atomic structure is defined by integer quantum numbers, restricted by an exclusion principle. Fission and fusion transmute the elements. However, the geometric periodism peculiar to organizing centres imposes on them a more complex hierarchy than the rows and columns of the Periodic Table.

Attempts are under way to synthesize some of the missing organizing centres, and to observe organizing centres in diverse excitable media. Solitary scroll rings, at least, have been reported in chemically-active media^{4,32} and in thick cardiac muscle³⁸.

Organizing centres could be biologically significant. They emit waves of short period comparable with the absolute minimum attainable, the medium's refractory period. Because waves mutually annihilate in collisions, higher frequency sources eventually dominate slower ones, including normal pacemaker centres, thus usurping control. Although modified by the inhomogeneities of biological media, such organizing centres may be involved with the pathological waves of spreading depression and epilepsy⁹⁻¹², and of cardiac fibrillation and sudden death^{13-16,38,41}.

A.T.W. thanks the NSF for grant CHE-810322, the US Department of Energy and Los Alamos National Laboratory for hospitality and Cray-1 computer time in summer 1983, Mel Prueitt for GRAFIC software and instruction.

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ARTICLES

India-Eurasia collision chronology has implications for crustal shortening and driving mechanism of plates

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The motion of the Indian plate is determined in an absolute frame of reference and compared with the position of the southern margin of Eurasia deduced from palaeomagnetic data in Tibet. The $2,600 \pm 900$ km of continental crust shortening observed is shown to have occurred in three different episodes: subduction of continental crust, intracontinental thrusting and internal deformation, and lateral extrusion. The detailed chronology of the collision and plate reorganizations in the Indian and Pacific oceans supports the hypothesis that slab-pull is a dominant driving mechanism of plate tectonics.

THE collision between the Indian and Eurasian continents is considered to be one of the major tectonic events of Cenozoic time. It has resulted in ~2,000 km of north-south crustal shortening within continental Eurasia¹. Several models have been proposed for the mechanism of this shortening: continuous deformation, multiple continental underthrusting, eastward propagating extrusion or mosaic tectonics²⁻⁵. Convergent plate motion still occurs between India and Eurasia and is evidenced in particular by active tectonics beneath the Himalayas, in Tibet and in south-central Asia⁶⁻⁸.

The continental effects of the collision have been extensively studied, in particular during the 1980-83 French-Chinese cooperation programme in Tibet⁵⁻⁹. Geological or geophysical observations on land can only give bounds on the age of the initial collision. For instance, structural observations along the Yarlung Zangbo suture zone (YZSZ) in Tibet (in the vicinity of Lhasa) show the ophiolites to have been obducted before the end of the Eocene (before 40 Myr)⁹. Such a young upper bound is also indicated by petrological and geochronological data on igneous rocks in Tibet. Indeed, the youngest granodiorites observed near Lhasa are dated at 41 Myr (ref. 10) and thus may be consistent with the subduction of oceanic crust beneath Tibet until at least 45 Myr, assuming up to 4 Myr for the transit and cooling of magma in the crust. An older age for the initial collision has been proposed from geological evidence and sea-floor spreading data¹¹. It is suggested that it began, at least in the Ladakh area, before the middle Eocene (near 53 Myr).

On the other hand, only a few studies have analysed the consequences of the collision on plate tectonics. In an early analysis of seafloor spreading in the Indian ocean, Molnar and Tapponnier¹² interpreted a decrease in the rate of northward drift of India near 40 Myr as indicative of the India-Eurasia collision. Using more detailed data, this slowing of the Indian plate has been associated with the decrease in spreading rate on the Eastern Indian ridge near anomaly 22 time (~50 Myr)¹³⁻¹⁵. Following Molnar and Tapponnier¹², several authors have related this change in spreading rate to the collision

between the two continental masses^{16,17}. However, the change in India-Africa relative motion observed at anomaly 20 (44 Myr) has been considered elsewhere as indicative of this collision¹⁷. Also, evidence for an important plate reorganization in the north-eastern Indian Ocean after anomaly 20 time has recently been put forward and interpreted as a consequence of the collision¹⁸.

Here we use a more comprehensive set of marine magnetic anomalies in the central Indian Ocean, in the vicinity of the triple junction¹⁹ to determine the detailed motion of the Indian plate since anomaly 32 time (late Upper Cretaceous) and to clarify the chronology of the collision. The relative position of India with respect to Eurasia is computed using a procedure originally proposed by Patriat *et al.*¹⁷. It is then compared with its absolute position with respect to the hotspot frame of reference. This latter absolute motion is compared with recently determined palaeomagnetic poles in Ladakh, Tibet and Thailand^{1,20-22} in order to determine the northern extent of India before the collision. The present data are combined with a recent interpretation of the northeastern Indian Ocean¹⁸ to provide a complete reconstruction of the Indian plate before, and immediately after, the India-Eurasia continental collision. The relative importance of the driving forces of plate tectonics is then discussed taking into account these reconstructions.

Age of the India-Eurasia collision

In the past 15 yr, an extensive survey has been performed in the Indian Ocean using ships *Gallieni* and *Marion Dufresne* (see ref. 23). This survey has allowed a detailed interpretation of marine magnetic anomalies in the Central Indian Basin, the Crozet Basin and the Madagascar Basin^{19,23} (Fig. 4b). These anomalies were generated at the accreting boundaries between the India, Antarctica and Africa plates, which meet at the Rodriguez triple junction. The simultaneous study of seafloor spreading on both flanks of these three ridges in the vicinity of the triple junction has allowed a complete and consistent

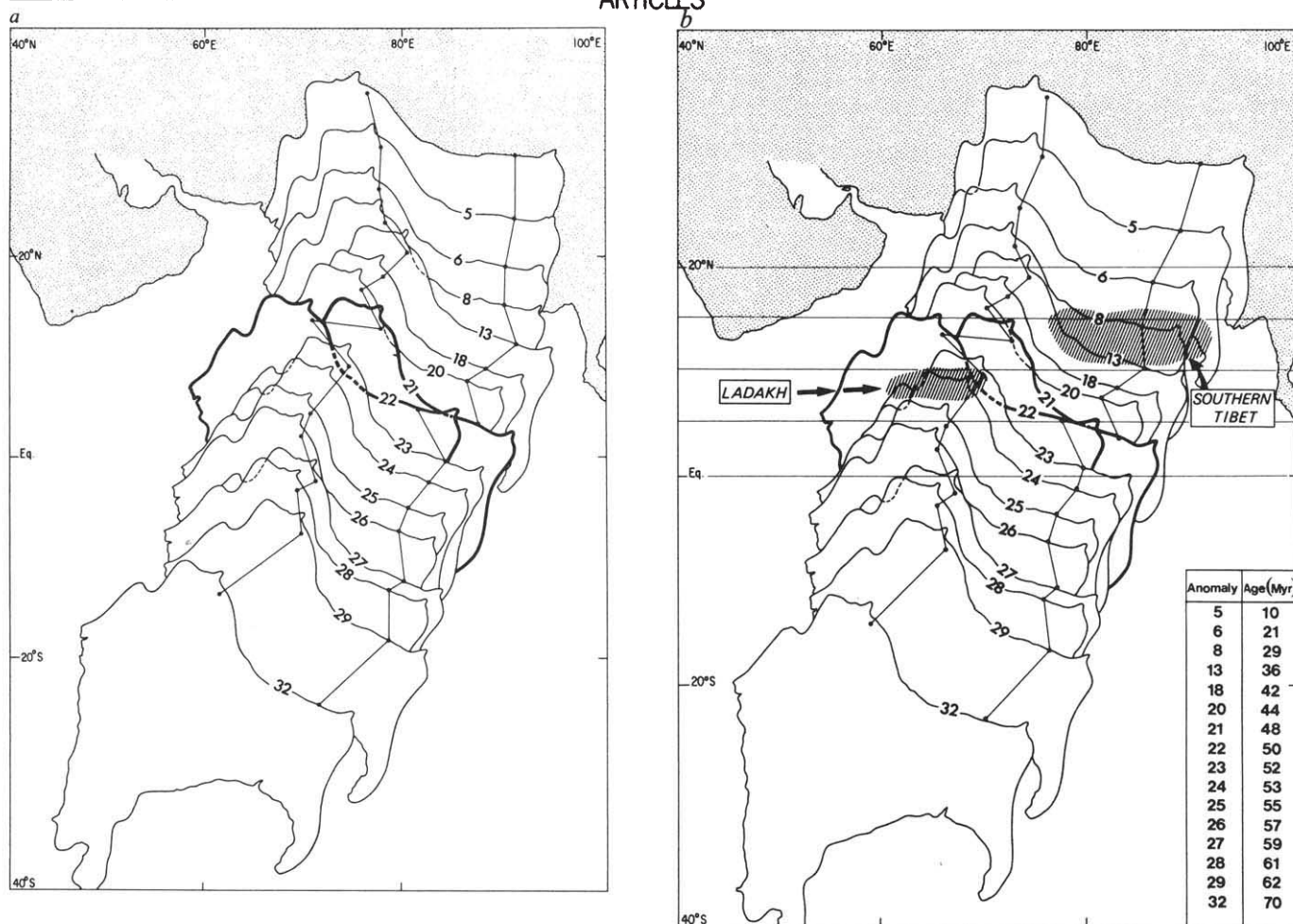


Fig. 1 Cenozoic northward drift of India. The northern limit of the Indian continent is taken as the present position of the Indus-YZSZ. The passive margins are drawn at the 500-m isobath (represented at anomaly 32 time). Also plotted is the motion of two points located on the Indian continent near the northern margin: in Ladakh ($34^{\circ}34' \text{ N}$, $76^{\circ}7' \text{ E}$) and on the suture zone near the present position of Lhasa ($29^{\circ}20' \text{ N}$, 91° E). The position of the northern margin of India is emphasized at the time of initial collision with the southern margin of Eurasia (anomalies 22 and 21). *a*, Relative motion of India with respect to Eurasia, kept arbitrarily fixed in its present position. *b*, Absolute motion of India in the hotspot frame of reference. The positions of southern Tibet and Ladakh before collision deduced from palaeomagnetic data, are also indicated for comparison.

reconstruction of the relative motion of the plates from anomaly 34 time (83 Myr) to the present¹⁹.

Figure 1*a* represents the successive positions of India since anomaly 32 time (70 Myr) with respect to the Eurasian plate which is kept arbitrarily fixed in its present position (Table 1). The position of India at a given time is obtained by combining the rotation of India with respect to Africa, determined from magnetic anomalies in the Indian Ocean¹⁹, with the rotation of Africa with respect to Eurasia, deduced from magnetic anomalies in the Atlantic Ocean²⁴⁻²⁶. Figure 1*b* shows the successive positions of India with respect to the hotspot frame of reference (Table 2). Here, India is, as before, first restored in its position with respect to Africa but then Africa is rotated in its position with respect to the hotspot reference frame at a given time²⁷.

The two reconstructions of Fig. 1 are very similar. It confirms that during the Cenozoic, the Eurasian plate underwent only a small clockwise rotation with respect to the Earth's mantle. This is evidenced by the apparent polar wander path (APWP) of this plate between 60 Myr and the present²⁸⁻³⁰ and also by hotspot tracks analysis²⁷. In addition, the location of the Euler pole for this rotation implies very little latitudinal displacement of the plate in the vicinity of the collision zone^{1,30}.

One can observe on Fig. 1 three successive phases in the drift history of India. First, down to anomaly 23 (52 Myr) India was drifting northward with a mean velocity of 15 to 20 cm yr^{-1} . Then, between anomaly 23 and anomaly 13 (36 Myr) the motion

of India became rather erratic showing several changes in direction while the mean northward velocity was reduced to $<10 \text{ cm yr}^{-1}$. Finally, from anomaly 13 to the present, India resumed a stable northward direction of convergence with respect to Eurasia with a constant rate of $<5 \text{ cm yr}^{-1}$.

The decrease of the mean velocity of India is seen in Fig. 2 where the rate of convergence between India and Eurasia is computed at two arbitrary points and plotted as a function of time, assuming two particular orientations of the plate boundary (subduction). Before anomaly 22 (50 Myr), the rate varied between 15 and 25 cm yr^{-1} ; it then decreased until anomaly 18 (42 Myr) (or 13, depending on the orientation of the trench), where it reached a stable value of $\sim 4 \text{ cm yr}^{-1}$.

The sudden velocity drop after anomaly 22 is more clearly observed directly on the evolution of spreading rates at the eastern and central Indian ridges (Fig. 3). Indeed, on both spreading centres bordering the Indian plate, one observes a sharp decrease in spreading rate between anomalies 22 and 21. On the central Indian ridge, the velocity decrease seems to have occurred slightly later in the vicinity of the Vema fracture zone (Fig. 3*a*). This should be attributed to the uncertainties in the reconstruction of plate motion in this area, also suggested by the frequent changes in the azimuth of spreading near anomaly 22 time (Fig. 3*b*) and due to the proximity of the pole of rotation.

These changes in the direction of spreading coincide with the surprisingly erratic behaviour of India, indicated in Fig. 1, at the time of anomalies 22 and 21. The Indian plate seems to have

Table 1 Euler poles of India-Eurasia relative motion during the Cenozoic

Age (Myr)	Anomaly	Lat. (°N)	Long. (°E)	Angle (°)
10.21	A5	16.6	37.6	7.44
20.98	A6	18.5	33.3	12.85
29.22	A8	16.9	34.8	17.74
36.47	A13	13.3	40.7	23.82
41.81	A18	17.7	37.0	26.98
44.40	A20	20.7	34.0	28.44
47.69	A21	16.0	35.0	33.27
49.68	A22	26.0	24.0	30.50
51.62	A23	18.1	28.7	36.44
53.03	A24	19.0	25.8	38.37
55.25	A25	20.1	22.1	40.81
56.92	A26	19.8	20.7	43.17
59.31	A27	16.2	22.7	48.67
60.57	A28	17.3	20.6	49.47
62.49	A29	14.9	21.5	54.65
70.62	A32	18.8	13.3	60.72

The rotation at A32 between India and Africa is obtained by combining the India-Antarctica and Antarctica-Africa rotations.

Table 2 Euler poles of India absolute motion during the Cenozoic

Age (Myr)	Anomaly	Lat. (°N)	Long. (°E)	Angle (°)
10.21	A5	27.7	31.1	7.83
20.98	A6	30.6	24.9	13.93
29.22	A8	28.6	26.0	19.13
36.47	A13	24.1	32.0	24.74
41.81	A18	26.8	29.2	27.51
44.40	A20	29.1	26.5	28.80
47.69	A21	23.0	28.7	32.50
49.68	A22	33.2	16.1	30.59
51.62	A23	24.2	22.4	35.22
53.03	A24	24.5	19.3	36.91
55.25	A25	25.2	14.9	39.30
56.92	A26	24.6	13.3	41.48
59.31	A27	20.2	15.7	46.18
60.57	A28	21.1	13.2	47.01
62.49	A29	18.1	14.2	51.52
70.62	A32	20.6	3.4	57.53

The rotation at A32 between India and Africa is obtained by combining the India-Antarctica and Antarctica-Africa rotations.

undergone, during this period, several successive important shifts in its direction of motion (absolute or relative to Eurasia). Since the momentum of a plate can always be neglected³¹, such shifts must be explained by successive changes in the balance of forces applied to the plate. The time constant for these changes is too short to allow a significant modification of the body or surface forces acting on the entire plate like gravitational sliding (ridge-push) or viscous drag. Thus, the shifts observed should rather be related to modifications at the plate boundaries. Following earlier studies, we suggest that the directional shifts and velocity decrease of the Indian plate observed at anomalies 22 and 21 time are likely to be associated with the closure of the Neotethys to the north of India and thus to indicate the onset of the collision of India with the southern margin of Eurasia.

Reconstructions based on magnetic anomalies 22 and 21 on both sides of the central Indian ridge are subject to some uncertainties. However, anomaly 21 is also well identified on the two flanks of the eastern and western Indian ridges, and the rotation of India with respect to Africa determined on the central Indian ridge is in close agreement with the combined rotation of India with respect to Antarctica and of Antarctica with respect to Africa. This confirms that even when uncertainties are taken into account, the shifts in plate motion direction described above cannot be avoided in the reconstructions. Thus, although a precise description of the actual motion of India between anomalies 23 and 21 time cannot be achieved, the shifts observed must be considered as significant and indicative of a peculiar and major event, such as the beginning of a continental collision.

Palaeomagnetic data in Tibet

In Fig. 1b, the position of the northern margin of India in the hotspot frame of reference is compared with the palaeoposition of the southern margin of Eurasia deduced from palaeomagnetic studies. It has been shown that the two frames of reference used for this comparison, the axial geomagnetic dipole field and the hotspot frame, have remained fixed within 5°–10° of each other, in the past 60 Myr (refs 27, 29). Furthermore, the consistency between the palaeomagnetically-determined latitude of India and its absolute position derived from oceanic magnetic anomalies and hotspot track analysis is confirmed by recent palaeomagnetic work in Tibet, on the northern margin of India²². This study of Eocene limestones, sampled to the south of the YZSZ, reveals two different components of magnetization. A primary component of Lower Eocene age indicates a palaeolatitude for the northern margin of India, in the vicinity of Lhasa,

of $2.5^\circ \pm 4^\circ$ S. Our present reconstruction places the sampled area at anomaly 26 time (the age of the magnetization) at a palaeolatitude of about 6° S (Fig. 1b). A secondary post-collisional component is also revealed by the study of Besse *et al.*²² and indicates a palaeolatitude of $6^\circ \pm 5^\circ$ N. This is again very consistent with our reconstructions which show the collision to have occurred in the area of Lhasa near 5° N (Fig. 1b).

Two palaeomagnetic poles have been derived for southern Tibet in the area of Lhasa for late Cretaceous and Palaeocene time¹. Southern Tibet was accreted to the southern margin of Eurasia before the late Jurassic⁵ and, therefore, indicates the position of this margin since that time. The late Cretaceous pole, derived from Albian-Aptian red beds, places the margin near Lhasa at a palaeolatitude of $11.5 \pm 3^\circ$ N. The early Cenozoic pole has a slightly larger confidence limit associated with the large timespan of volcanic emplacement (ages range at least

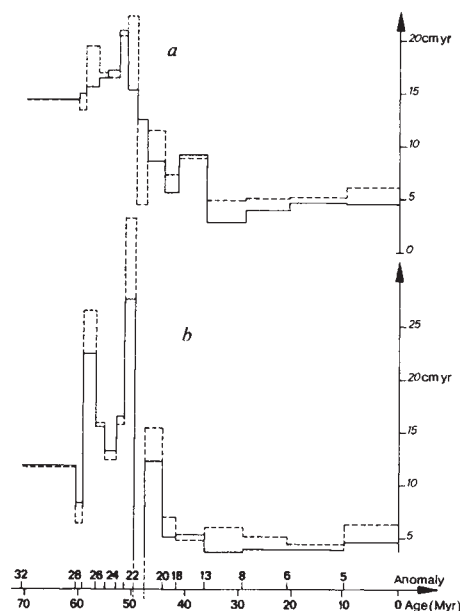


Fig. 2 Computed rate of convergence between the Indian and the Eurasian plates during the Cenozoic, at two arbitrary points (5° N, 75° E, solid line; 5° N, 90° E, dashed line) along two particular directions. It indicates the rate of subduction perpendicular to the trench for a subduction zone located near the southern margin of Eurasia and trending N120 E (a) or east-west (b).

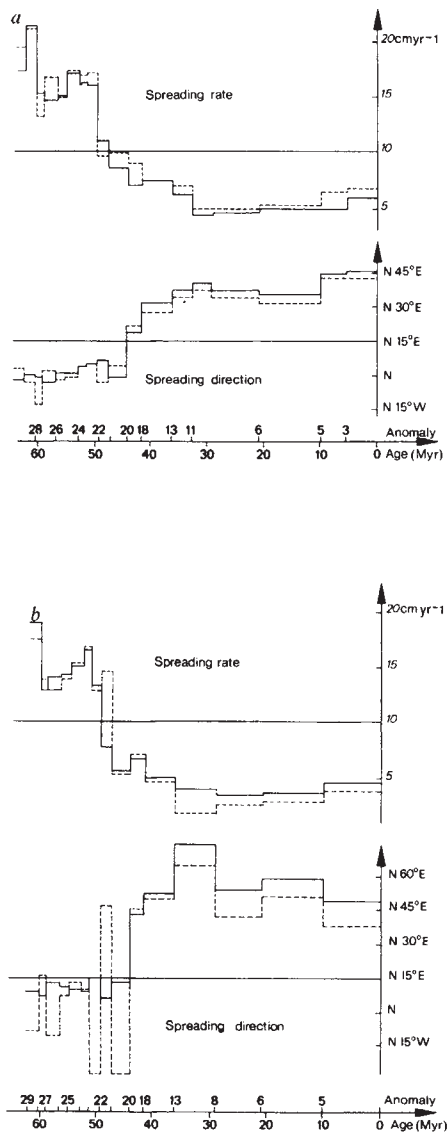


Fig. 3 Measured rate and direction of spreading at two accreting boundaries of the Indian plate during the Cenozoic. *a*, Eastern Indian ridge, in the vicinity of the Rodriguez triple junction (solid line) and near the Amsterdam fracture zone further east (dashed line). *b*, Central Indian ridge, in the vicinity of the triple junction (solid line) and near the Vema fracture zone further north (dashed line). The important shifts in spreading direction near the Vema fracture zone are related to the proximity of the reconstructed poles of rotation at the time of the collision (anomalies 22 and 21).

from 60 Myr (ref. 32) to 48 Myr (ref. 33)), secular variation of the Earth's magnetic field and the possible occurrence of Cenozoic deformation within southern Tibet^{1,34}. This younger pole indicates a palaeolatitude of $13 \pm 6.5^\circ$ N for the southern margin of Eurasia.

Another palaeomagnetic pole has been derived in Tibet, north of the Indus–YZSZ, for Palaeocene–Eocene time in the Ladakh area²⁰. The Ladakh intrusives have been interpreted as the batholithic foundation of an island arc^{35,36}. The remanent magnetization recorded in these intrusives indicates a palaeolatitude between 7 and 10° N and is considered to have been acquired shortly before 50 Myr.

It can be seen on Fig. 1*b* that the northern margin of India collided with the Ladakh island arc before anomaly 24 (53 Myr).

It is not until anomaly 22 (50 Myr), however, that the first significant change in the direction of motion occurred on the Indian plate. This confirms that such a collision between a large continent and an offshore island arc is unlikely to significantly alter the motion of the plate.

In contrast, one observes on Fig. 1*b* that at anomaly 22 time, the northern margin of India near Lhasa still lay about 7° south of the southern margin of Eurasia (southern Tibet). Since Argand³⁷, several estimates of crustal shortening have been proposed for the Indian continent and accounted for, either by continental subduction beneath Tibet along the YZSZ, or by underthrusting within India (along the Main Central thrust (MCT), Main Boundary thrust (MBT) or Kangmar thrust. See, for example, refs 5, 38). The reconstruction proposed here implies between 400 and 1,000 km of continental shortening between India and southern Tibet, at the longitude of Lhasa. Uncertainties are taken from the position of India at anomalies 23 and 20 (see Fig. 1) and are thus likely to be overestimated.

Driving mechanism of plates

The analysis of seafloor spreading on the central and eastern Indian ridges reveals that the decrease in spreading rate at anomaly 22 is followed at anomaly 20 by a consistent and significant change in the direction of spreading (Fig. 3). A more detailed analysis of magnetic anomalies in the Central Indian Basin and the Crozet Basin reveals that this change in spreading direction did not occur synchronously along the entire eastern Indian ridge. Instead, the change seems to have propagated eastward along the ridge. Anomaly 20 is parallel to the new direction of spreading near the triple junction but is still parallel to older anomalies further east¹⁹. The change in spreading direction on the central Indian ridge was accompanied by a jump of the spreading centre. This is evidenced by the existence in the Madagascar Basin, near Ile Maurice, of a fossil spreading centre which ceased spreading just before anomaly 20 time¹⁹.

Liu *et al.*¹⁸ have re-analysed magnetic anomalies in the north-western Wharton Basin, south of Sumatra. They have recognized a fossil spreading centre. The existence of this fossil ridge was first reported by McDonald³⁹ who observed a series of basement highs beneath the sedimentary cover of the Nicobar Fan. This accreting plate boundary which separated India from Australia in the early Cenozoic, before the India–Eurasia collision, ceased its spreading shortly after anomaly 20 time¹⁸.

Cande and Mutter⁴⁰ have re-identified the oldest magnetic lineations between Australia and Antarctica. They propose that initial rifting between the two continents took place between 110 and 90 Myr but seafloor spreading occurred at a very low rate of <1 cm yr⁻¹ until anomaly 21, where it reached a value of 2 cm yr⁻¹. After anomaly 20, this spreading rate suddenly increased significantly to a value of 5.5 cm yr⁻¹.

All these observations indicate the occurrence of a global plate reorganization in the Indian Ocean which seems to have started near the Indian triple junction immediately before anomaly 20, near 45 Myr, and to have been completed before the formation of anomaly 19, near 43 Myr. Figure 4 represents the plate configuration in the Indian Ocean at anomaly 24 time (53 Myr), that is before the beginning of the collision (Fig. 4*a*), and at anomaly 18 time (42 Myr), when the plate reorganization had been completed (Fig. 4*b*). The reconstruction of the spreading centre between India and Australia is based on the new identification of magnetic anomalies in the Wharton Basin¹⁸. The central and western Indian Ocean are reconstructed from the identification of magnetic anomalies described in Patriat¹⁹. Finally, the whole reconstruction is rotated in an absolute position with respect to the hotspot frame of reference. This allows the comparison with palaeopositions deduced from palaeomagnetic studies.

The position of the southern margin of Eurasia is given by palaeomagnetic data in Tibet (see above) and Thailand. Mesozoic poles for the Indochina block indicate an early Tertiary palaeolatitude for this block between 15 and 20° N (ref.

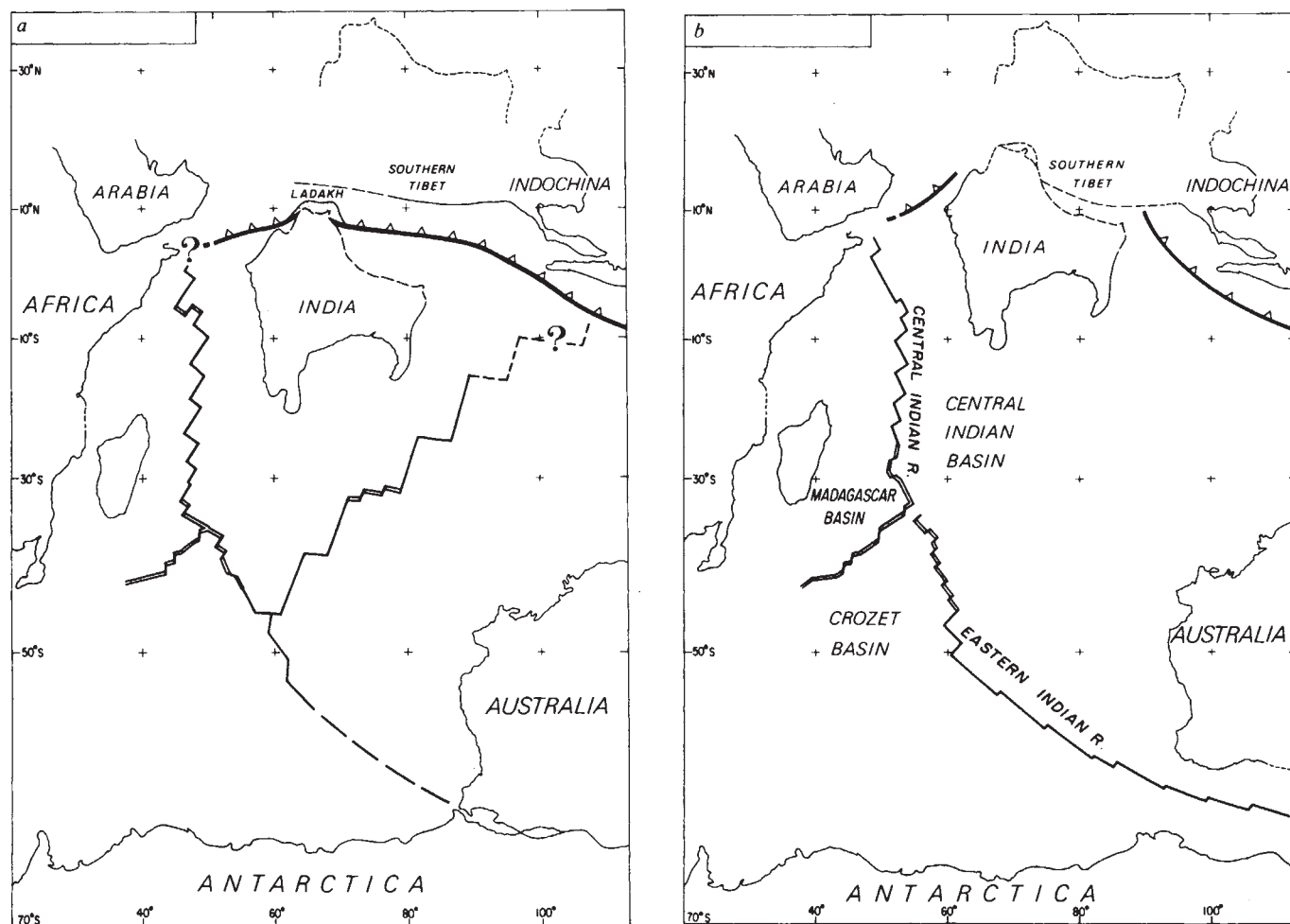


Fig. 4 Plate reconstruction of the Indian Ocean in an absolute frame of reference (hotspot and axial geomagnetic field), before the India-Eurasia collision (at anomaly 24 time, *a*) and immediately after the end of the plate reorganization which followed the collision (at anomaly 18 time, *b*). The contours of Arabia, Africa, Madagascar, Antarctica and Australia were digitized at the 2,500-m isobath. The contours of India were taken at the 500-m isobath (because of the thick series of deltaic sedimentary deposits) and along the Indus-YZSZ to the north (dashed line). The southern margin of Eurasia is reconstructed from palaeomagnetic data (southern Tibet, Ladakh and Indochina-Thailand). The present position of the Indus-YZSZ is also shown for comparison (dashed line). Double lines represent the position of spreading centres reconstructed from identified magnetic anomalies^{18,19}. The position of other spreading centres is more speculative and may be subject to revision. The subduction zone (barbed line) is located after the distribution of Upper Mesozoic granites in Tibet and southeastern Asia. Euler poles for the motion of Australia with respect to Antarctica are taken from ref. 51. The overlap in the reconstruction at anomaly 24 between Australia and Antarctica is consistent with the model of propagating rift and continental breakup of Courtillot⁵⁰.

21). This study also indicates an approximate 20° clockwise rotation of Indochina with respect to Eurasia since the collision, thus allowing Indochina to be restored to its pre-collision position (Fig. 4*a*). The subduction zone is located along the southern margin of Eurasia as suggested by the occurrence of Upper Mesozoic granites (see ref. 30). Although, as discussed above, ~700 km of crustal shortening is likely to have occurred within the Indian continent, the northern limit of India before the collision is indicated by the Indus-YZSZ in Fig. 4*a*.

The slab-pull force associated with subduction zones is a dominant force controlling the direction of motion of a plate^{41,42}. Indeed, the sequence of events associated with the collision and the plate reorganization are all very consistent with this hypothesis. First, as discussed above, the dramatic decrease of the Indian plate velocity at anomaly 22 time (50 Myr) is associated with the closure of the Neotethys in Tibet. This event affected >50% of the total length of subducting boundary of the plate (Fig. 4*a*). However, the northward motion of India continued until anomaly 20 time (44 Myr) when a new regime of plate tectonics was established in which India and Australia became a single plate. This 5-Myr delay between the initial contact (closure of the Neotethys) and this major plate reorganization corresponds, as seen in Fig. 1, to the subduction of

300–500 km of Indian continental crust beneath Tibet. This value is in agreement with earlier estimates of the amount of continental crust which can be subducted in a trench⁴³ suggesting that the plate reorganization did not occur until subduction had nearly stopped beneath Tibet, when the slab-pull force acting on the Indian plate along the colliding boundary was balanced by the buoyancy of the subducted continental crust. This is further indicated by the convergence rate between India and Eurasia which continues to decrease rapidly until anomaly 20 time and keeps a nearly constant values afterwards (Fig. 2).

As shown in Fig. 4*b*, India and Australia have become part of the same plate after anomaly 20 time. A counterclockwise rotation of India with respect to Eurasia is observed since that time (Fig. 1). This rotation is also indicated by palaeomagnetic studies on DSDP cores in the Indian Ocean⁴⁴ and on the Deccan Trapps in India⁴⁵ and is matched in southern Tibet by a similar counterclockwise rotation with respect to Eurasia¹. This is in agreement with the fact that the suturing of India against Eurasia had been completed by anomaly 20 time. The distribution of subducting boundaries bordering the new larger Indian plate, essentially the New Guinea and Java trenches, is consistent with the observed counterclockwise rotation in the late Cenozoic in the hypothesis of a driving mechanism dominated by slab-pull

forces. Furthermore, this rotation of the Indian plate is likely to imply the onset of a convergent relative motion at the India-Pacific plate boundary. Indeed, one observes the creation in the early Tertiary of several thousands of kilometres of new subduction zones along the southwestern boundary of the Pacific plate (Tonga-Kermadec, Macquarie; see refs 41, 46). The bend observed in the Hawaiian-Emperor seamount chain has been interpreted as evidence for a change in the absolute motion of the Pacific plate and shown to be consistent with the redistribution of slab-pull forces acting on this plate⁴¹.

The bend in the Hawaiian-Emperor chain occurred near 42 Myr. Thus, the time lag between the onset of a convergent motion between the two plates (the end of the plate reorganization in the Indian Ocean) and the creation of new slabs acting on the plate (and producing the change in the direction of plate motion evidenced by the bend) is of the order of 2 Myr. McKenzie⁴⁷ has shown that the creation of a new trench may be considered as a finite amplitude instability which develops in an elastic plate only for a horizontal convergence greater than 180 km (for a slab dipping at 45°). For a typical relative plate velocity of 10 cm yr⁻¹, the 2-Myr time lag observed corresponds to a horizontal convergence of the order of 200 km.

The observed chronology of events during the plate reorganization also suggests that ridge-push associated with the thermal structure of plates is not a dominant driving force and that seafloor spreading is a passive phenomenon. Indeed, the change in spreading direction of the Indian plate seems to occur in the vicinity of the triple junction before spreading ceased in the eastern Indian basin. The new direction of spreading on the central and eastern Indian ridges tends to be more parallel to the ridge between India and Australia, and thus, the termination of spreading at this ridge seems to be a consequence rather than a cause of the plate reorganization.

Crustal shortening mechanisms

Palaeomagnetic studies in Tibet have shown the existence of 1,900 ± 850 km of shortening within Eurasia since the collision¹. The present comparison of palaeomagnetic data and reconstructions based on magnetic anomalies document an additional 700 ± 300 km of convergence between India and southern Tibet. Thus, the total north-south shortening resulting from the collision between India and Eurasia amounts to 2,600 ± 900 km.

The last shift observed on India's motion occurred at anomaly 13 time (Fig. 1). This could be regarded as an artefact of our reconstructions since the finite rotations for the closure of the Atlantic Ocean were computed at anomalies 24 and 13 (see ref. 17). However, this shift appears both in the motion of India relative to Eurasia (Fig. 1a) and relative to the hotspot frame of reference (Fig. 1b). It thus seems to indicate an actual change in the motion of the Indian plate. The motion observed between anomalies 18 and 13 corresponds to several hundred kilometres of left lateral strike-slip motion between India and Eurasia in addition to north-south shortening. Tectonic studies in Tibet have shown the frequent occurrence of a large component of strike-slip motion along thrust faults and suture zones⁵ (YZSZ, Bangong-Nujiang suture, Yangbajain thrust, MCT). In addition, palaeomagnetic studies have shown internal deformation to occur in Tibet, in the vicinity of large thrust faults^{1,34}. The present observations suggest that strike-slip motion and penetrative deformation may have been contemporaneous with reverse faulting during the early phase of the collision.

After anomaly 13, transverse motion is no longer observed and >1,500 km of shortening still occurred within Eurasia while India moved steadily northward and rotated counterclockwise¹. This change near anomaly 13 may be interpreted as a change in the mechanism of shortening of Eurasia. The oldest magnetic lineations in the South China Sea have been identified as anomaly 11 (32 Myr)⁴⁸. Following Courtillot⁴⁹, we interpret the magnetic quiet zone as the consequence of continental rifting. Initial rifting is thus likely to have begun near anomaly 13 in the South China Sea. Tapponnier *et al.*³ have related this rifting

with the occurrence of left lateral strike-slip motion along the Red River fault which allowed the rotating extrusion of the Indochina block out of the way of the Indian indenter.

Thus, we suggest that the crustal shortening which followed the collision occurred in three episodes. First, from the initial collision to anomaly 20, say from 50 to 44 Myr, shortening occurred mainly by subduction of ~400 km of Indian continental crust beneath southern Tibet. This initial phase terminated when the buoyant resistive force opposed the subduction. Then, from anomaly 20 to anomaly 13 time, between 44 and 37 Myr, shortening continued essentially through crustal thickening by continental underthrusting and internal deformation. During this second phase, left lateral strike-slip motion was also taking place and the total associated north-south shortening is of the order of 300 km. Finally, since that time, shortening within Eurasia has been mostly taken up by block rotation and lateral extrusion along large left-lateral strike-slip faults as proposed by Tapponnier *et al.*^{3,4}. This last phase was initiated by activation of the Red River fault and associated rifting in the South China Sea.

Of course, the three mechanisms must have overlapped. In particular, during the last phase, lateral extrusion occurred first along the Red River fault shifting afterwards to the Altyn Tagh fault. During the transition the two other shortening mechanisms must have been active to allow the northward movement of India. In addition, seismic observations indicate that underthrusting is currently observed south of the Himalayas (MBT). However, the present data seem to indicate that during each episode, one particular mechanism dominated. Therefore, if we assume that during the third phase some 80% of the shortening results from lateral extrusion, this mechanism accounts for more than 1,500 km of north-south convergence of India and Eurasia. Because the two other mechanisms account for ~1,100 km of the total shortening, lateral extrusion appears to have been the most efficient in deforming the continent.

Conclusion

The analysis of Cenozoic magnetic anomalies in the Indian Ocean confirms that the collision between India and Eurasia began at anomaly 22 time, that is 50 Myr ago. This age is consistent with all previously available bounds given by geological or geochronological observations. In particular, it is very consistent with the age of volcanics in the Lhasa area. A K-Ar study on an ignimbrite indicates an age of 48.5 ± 1.5 Myr (ref. 33). Also, several ³⁹Ar/⁴⁰Ar analyses on andesites show an important grouping of ages at 50 Myr (ref. 50). The collision with the Ladakh island arc probably occurred earlier, near 54 Myr, but it does not seem to have affected the motion of the Indian plate.

A comparison with palaeomagnetic data in southern Tibet (the southern margin of Eurasia before the collision) shows that 'greater' India extended between 500 and 1,000 km further north than the present northern limit of the Indian continent (the YZSZ). The total continental shortening of India and Eurasia produced by the collision is 2,600 ± 900 km and seems to have occurred in three successive phases.

The termination of subduction beneath Tibet at 44 Myr (anomaly 20 time) produced a change in the direction of motion of the Indian plate. This resulted in the termination of spreading between India and Australia which became attached, and a sudden increase in the spreading rate between Australia and Antarctica^{18,40}. This plate reorganization in the Indian Ocean preceded the shift in the direction of absolute motion of the Pacific plate evidenced by the bend of the Hawaiian-Emperor chain. This shift may therefore be interpreted as a consequence of the India-Eurasia collision. Finally, the relative chronology of this plate reorganization, which is well constrained by the present study, is very consistent with the hypothesis that the slab-pull force associated with subduction zones is a dominant driving mechanism of plate tectonics.

This work has benefited from many discussions with C. Jaupart. This is IGP contribution no. NS 776.

Received 1 May; accepted 25 July 1984.

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Palaeomagnetic estimates of crustal shortening in the Himalayan thrusts and Zangbo suture

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This article reports new palaeomagnetic results from precisely-dated late Palaeocene limestones from the former northern margin of the Indian subcontinent, now in Tibet, between the major Himalayan thrusts and the India–Tibet suture zone. Convergence in the suture and intracontinental thrusting can be estimated separately and are respectively found to be of the order of 550 km (± 650 km) and 400 km (± 400 km). Comparison with kinematic models derived from magnetic anomalies constrains the age of the collision at about 50 Myr.

CRUSTAL shortening in major orogenic belts involves, in addition to folding, one or several modes of deformation: subduction of continental crust in the wake of pre-existing oceanic subduction, and sideways escape of crustal blocks along major strike-slip faults (see refs 1–4). A direct measurement of the amount of shortening is often not possible and the relative contributions of each mode have remained highly controversial, particularly in the case of the world's largest orogen, the Himalayan range and associated Tibetan plateau^{5–9}. Palaeomagnetism remains the only indirect means of obtaining quantitative estimates.

Continental collision of India with southern Tibet, at that time the southern active margin of Eurasia, occurred in the late Palaeocene–Eocene. The rough original estimate of the age of collision was based on the drastic change in relative motions observed in magnetic anomalies from the Indian Ocean¹⁰. Large-scale continental shortening followed, firstly, along the original Indus–Zangbo suture zone and then to the south of the suture, along newly developed major continental thrusts such as the Main Central thrust (MCT) and Main Boundary fault (MBF) south of the High Chain, or the recently identified Kangmar thrust (KT) to the north of the High Chain^{3,11}. The amount of convergence along the suture remained essentially unknown, and estimates of convergence along the intracontinental thrusts were a cause of great controversy, ranging from 200 km or less⁸ to >1,500 km (ref. 6 and see also refs 5, 7, 9). A study of middle Cretaceous red beds and Palaeocene–Eocene volcanics from southern Tibet (Lhasa Block) has recently constrained the total amount of post-80 Myr (and, with some uncertainty on age,

post-60–50 Myr) convergence between India and southern Tibet to 1,400 $\pm$ 900 km (ref. 4).

Geological setting

During the first (1981) and the third (1983) French–Chinese geoscience expeditions to Tibet, we sampled an outcrop of early Cenozoic shallow marine sediments deposited on the northern margin of India (Fig. 1). The series starts with grey limestones rich in foraminifera (*Nummulites*, *Orbitolites*) and dated as Thanetian to Illeridian (that is between 50 and 60 Myr, ref. 12). The shallow water depositional environment is clearly demonstrated by algae and polyps. The limestones are conformably overlain by reddish fine-grained sandstones, witnessing the progressive rise of the Indian margin above water level. The entire sequence lies conformably over Cretaceous limestones and shales and appears to be analogous to the Spiti sequences in Ladakh¹³; it is folded in a large synclinalorium, trending 110° N and verging south (Fig. 2). The entire unit is located structurally to the north of the MBF, MCT (and High Chain) and to the south of the KT and Zangbo suture (Fig. 1).

Age determination and palaeomagnetic results

Oriented hand samples were taken near Dingri (Figs 1, 2) in limestones (sites XPP 24, 25; XPB 62, 63) and sandstones (site XPB 60). Accurate biostratigraphical age determinations were made by Feinberg, ages and faunas being scaled to the Contessa section in Italy¹⁴. Deposition of the limestones was found to

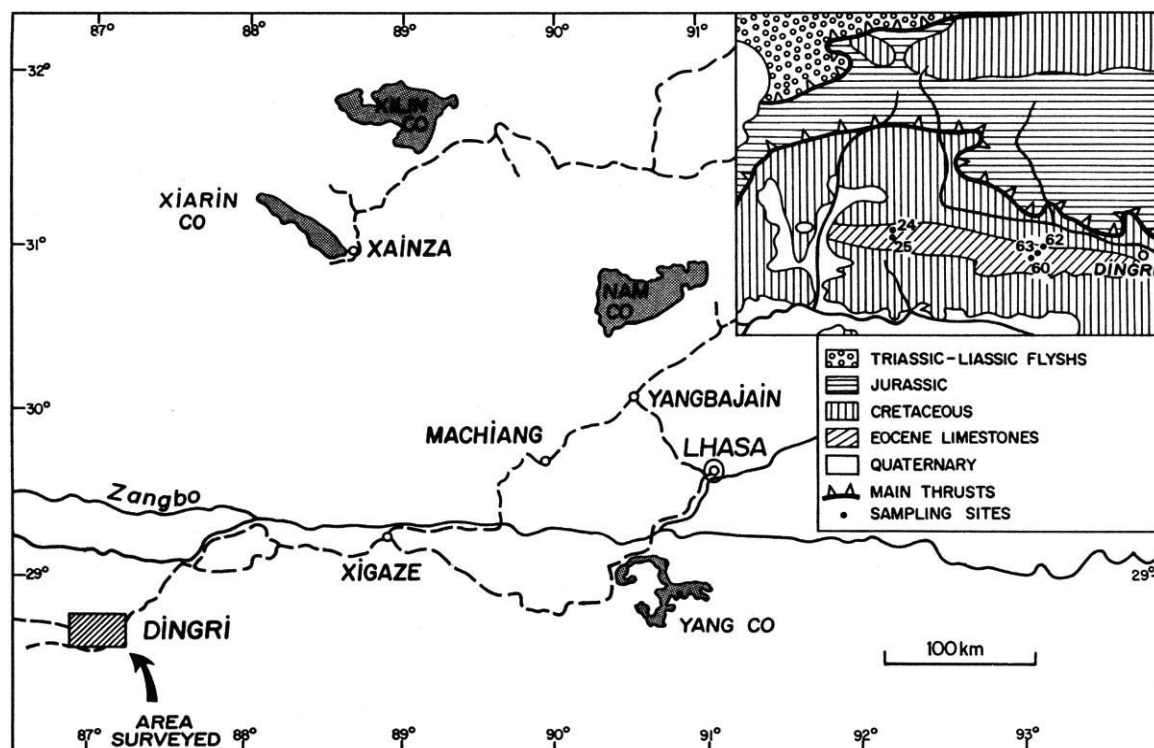


Fig. 1 Geographical map of the sampling area and schematic geological map (inset, after refs 11, 13).

have occurred strictly between anomalies 26 and 25. A comparison of various magnetic polarity time scales¹⁴⁻¹⁶ leads to an age of 57 ± 1 Myr. Specimens were cored from the samples in the laboratory and demagnetized by step-heating followed by cooling in approximately field-free space (20 nT), or by using alternating fields (AF). Magnetic measurements were done with a two-component CTF cryogenic magnetometer. Most samples had weak multicomponent magnetizations which were separated with a modified version of Kirshvink's principal component analysis¹⁷.

Only three limestone sites provided reliable directions. The magnetization of specimens from site 25 fell below $5 \times 10^{-6} \text{ A m}^{-1}$ at 200 °C. Typical vector demagnetization plots are given in Fig. 3. Two distinct components (A and B) are clearly indicated for the limestone specimens. Component B disappears after heating at 250–300 °C. The mean direction for sites 24, 62 and 63 is declination, $D = 14^\circ$, inclination $I = 11.5^\circ$ ($\alpha_{95} = 11.5^\circ$) before bedding correction. This is significantly different from either the present geomagnetic direction ($D = 0^\circ$, $I = 43^\circ$) or the dipole direction ($D = 0^\circ$, $I = 48^\circ$) at the sampling sites (lat. 29° N , long. 87° E). Applying the bedding correction increases the

scatter of the mean direction. This indicates a secondary (post-folding) remagnetization. Note that the points do not follow a Fisherian distribution: the scatter in declination is approximately twice the scatter in inclination (Fig. 4). Component A has unblocking temperatures between 300 and 500 °C and the demagnetization path points to the origin (Fig. 3). The principal carrier of remanence A is thus probably a (titano-) magnetite. Both normal and reverse polarities are observed and a positive fold test is obtained (Table 1, Fig. 4). The resulting mean direction $D = 337.6^\circ$, $I = -4.5^\circ$ ($\alpha_{95} = 14.6^\circ$) is thus pre-folding and probably of primary origin.

The single sandstone site (no. 60) also showed a multicomponent magnetization, with a more complicated behaviour. A scattered component with high-unblocking temperatures (500–640 °C), probably carried by haematite, is close to the A component of limestones after application of a bedding correction (Table 1: $D = 339^\circ$, $I = -10^\circ$, $\alpha_{95} = 24^\circ$). Beyond 620–640 °C, the progressive build-up of viscous remanent magnetization prevents a close study of the final steps of demagnetization. Depending on the lithology of the samples, two components with medium-unblocking temperatures were observed. One, found

Table 1 Palaeomagnetic results

	Component A								Component B								Component C											
	Corrected for bedding				Uncorrected				Corrected				Uncorrected				Corrected				Uncorrected							
	<i>N</i>	% <i>n</i>	<i>D</i>	<i>I</i>	<i>D</i>	<i>I</i>	α_{95}	<i>N</i>	% <i>n</i>	<i>D</i>	<i>I</i>	<i>D</i>	<i>I</i>	α_{95}	<i>N</i>	% <i>n</i>	<i>D</i>	<i>I</i>	<i>D</i>	<i>I</i>	α_{95}							
Site 24	5	100	335	−9	335	11	16	17	100	20	−20	20	17	7														
Site 25	No characteristic direction																											
Site 60	6	50	329	−10	323	30	24	7	0	29	−22	27	13	13	7	100	313	−47	328	−2	7.5							
Site 62	10	23	344	−9	338	41	10	11	30	16	−41	13	10	22														
Site 63	7	15	334	4.5	310	−46	17	5	15	15	77	19	7	17														
Site mean	<i>D</i> = 335.5, <i>I</i> = −5.9				<i>D</i> = 327.3, <i>I</i> = 11				<i>D</i> = 20.7, <i>I</i> = −8.0				<i>D</i> = 17.2, <i>I</i> = 11.8															
	<i>K</i> = 76, α_{95} = 10.6				<i>K</i> = 4, α_{95} = 50.4				<i>K</i> = 3, α_{95} = 71.9				<i>K</i> = 84, α_{95} = 10.1															
																	Palaeolatitude											
																	λ = 6° ± 5°											
Mean C + A					<i>D</i> = 154.0°, <i>I</i> = 5.1°, <i>K</i> = 83, α_{95} = 8.4°, palaeolatitude = −2.5° ± 4°																							

N is the number of samples, % n is the percentage of samples of normal polarity.

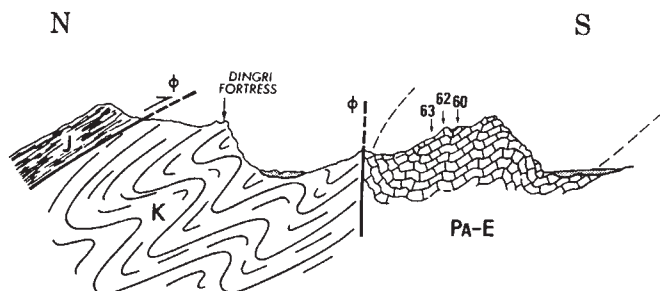


Fig. 2 Simplified cross-section going through sites 60-63. Site 60 is in sandstones, 62 and 63 are in limestones.

Table 2 New apparent polar wandering path for India and expected palaeolatitudes in Dingri (lat. 29° N, long. 87° E)

Age (Myr)	Anomaly no.	Pole lat. (°N)	Pole long. (°E)	Palaeolatitude in Dingri (°)
70	32	47.5	301	-22.5 (S)
62	29	41.2	293	-16
61	28	46.1	292.5	-12
59	27	46.6	294.5	-10
57	26	52	293	-6
55	25	55	293.5	-3
53	24	56.4	298	-0.6
52	23	58	300	1.3 (N)
50	22	64.6	294.3	6.3
48	21	66	306	4.6
44	20	64.4	303	7.2
42	18	64.8	303	8
36	13	67	308	11
29	8	73	301	14.5
21	6	77.7	300	18.5
10	5	83	304	23

between 120 and 450 °C, is close to component B before bedding correction (Table 1: $D = 27^\circ$, $I = 13^\circ$, $\alpha_{95} = 13^\circ$). More interestingly, a second component (C) is found to be $D = 328^\circ$, $I = -2^\circ$ ($\alpha_{95} = 7.5^\circ$) before bedding correction, which is close to component A after bedding correction. The medium-to-high unblocking-temperature components are also revealed by the converging circles method of Hoffman and Day¹⁸ and occur as (alternate) discontinuous linear segments on the vector diagrams, generating a characteristic 'Christmas-tree' pattern (Fig. 3). Both normal and reversed directions are found, often intermediate between directions A and B before and after bedding correction. Secondary components may have an unblocking-temperature spectrum that extends to high temperatures and may be responsible for the scatter of the high-temperature component. These observations (particularly component C), together with the scatter in declination of the B component of limestones, suggest that part of the (re)magnetization was acquired during folding, and part shortly after folding.

The combined high-temperature component A for all sites passes a positive fold test¹⁹ at the 99% level (Table 1). The previous discussion leads us to interpret it as being primary and to assign it the age of 57 ± 1 Myr. The depositional palaeolatitude at that time was then $2.5^\circ \text{S} \pm 4^\circ$, close to and slightly south of the Equator. On the other hand, the B component is a remagnetization, acquired at $6^\circ \text{N} \pm 5^\circ$, during the end or immediately after folding (the age of which is as yet unknown): the fold test is negative at the 99% level. The precise mechanism of overprinting remains unknown although it may be related to the growth of new magnetic minerals, induced by solution transfer under stress.

APWP of India

The determination of continental shortening in the MBT and MCT requires that our data be compared with palaeomagnetic data of similar age from the Indian shield. Such data are unfortu-

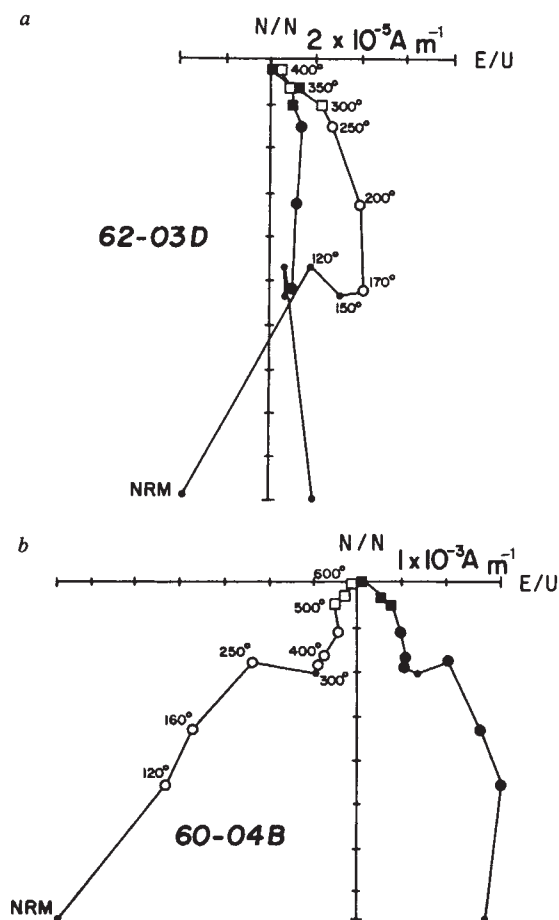


Fig. 3 Typical thermal demagnetization diagrams in geographical coordinates for *a*, a limestone (62-03D) and *b*, a sandstone (60-04B) specimen. Open symbols are for horizontal components, closed symbols are for the component in the north-south vertical plane. Circles are for the lower-temperature B component (remagnetization) and squares for the high-temperature (primary) A component.

nately not available. However, two indirect estimates of the Indian apparent polar wander path (APWP) are available. One determination is based on inclinations from DSDP cores²⁰, for which, however, no declination control is available. As a result, true palaeolatitudes are systematically underestimated²⁰. The lacking declination data are supplemented by magnetic anomaly data. The resulting APWP for India for the period 70-0 Myr (ref. 20) is shown in Fig. 5, together with error circles which are probably underestimates. We redetermined the 60 Myr pole, which is of particular importance here, by discarding highly suspicious results from DSDP sites 215 (basalts) and 217 (sediments).

Because a perfect calibration in age is fundamental here, a new Indian APWP (late Cretaceous and Cenozoic) can be derived on the basis of a detailed study of magnetic anomalies in the Indian Ocean²¹ combined with the 'absolute' motion of Africa (with respect to hotspots) deduced by Morgan²² (see also ref. 23). The new APWP is shown in Fig. 5 and the corresponding palaeomagnetic directions expected for Dingri are listed in Table 2. It is not easy to determine error bars for these estimates. Magnetic anomaly reconstructions contribute an error of the order of 2° (P. Patriat, personal communication, and refs 24, 25), which is not isotropic, and hotspot positions contribute $\sim 2^\circ$ (as estimated in ref. 25), yielding a total error of about 3° . On the other hand, the age control is excellent²¹.

The two APWP estimates are not significantly different after 50 Myr. From 50 to 70 Myr, they are systematically offset (rotated) one with respect to the other, by $\sim 10^\circ$. If this difference is significant, it may arise for several reasons, such as systematic

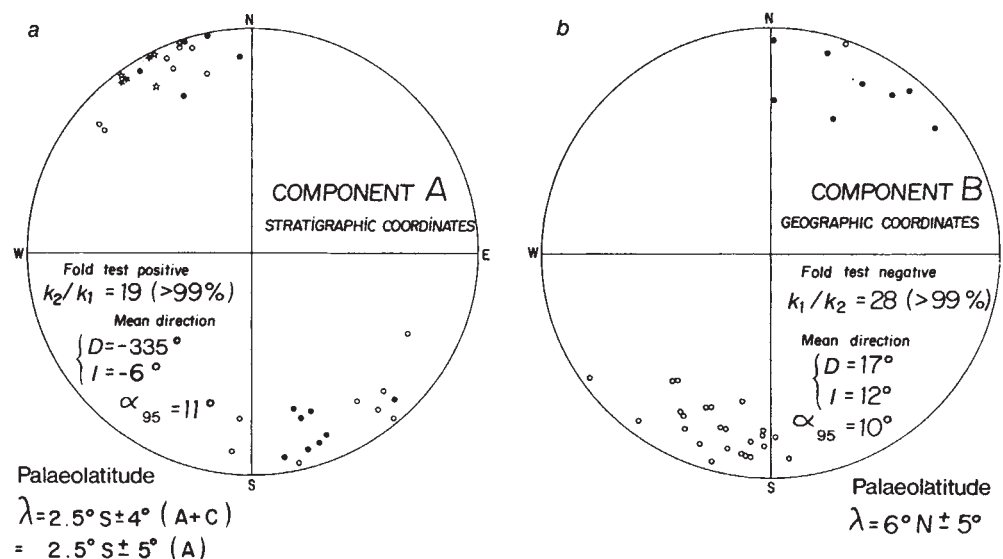


Fig. 4 Stable magnetic directions for all Dingri specimens. A and B (directions in stratigraphical coordinates) are shown as circles, C (in geographical coordinates) as stars (see text and Table 1).

differences between the geocentric axial dipole and hotspot reference frames²⁵ or more likely from errors in generating the DSDP curve. The only available 70 Myr Deccan traps palaeomagnetic pole²⁰ does not resolve this dilemma, as it falls about halfway between the two APWPs (Fig. 5).

Continental convergence and collision

We are now in a position to generate palaeolatitude against time plots for points of the northern margin of India (Fig. 6), namely the former northern edge of continental India, and points now located near Dingri and to the south of it before convergence on the MBF and MCT. Using the data of refs 21, 23, the new APWP for India implies $3.5^\circ \pm 4^\circ$ of convergence south of Dingri (Fig. 6). The DSDP path leads to a larger value of $5.5^\circ \pm 4.5^\circ$. These values are deduced from the 57-Myr old A component of magnetization. The error estimates are, as usual in palaeo-

magnetic studies, at the 2σ level and include errors on palaeomagnetic determinations and kinematic reconstructions. Further geological constraints help in reducing them: because we know that convergence definitely occurred between Dingri and India, the latitude difference cannot be negative or even smaller than $\sim 1^\circ$, the smallest possible amount of underthrusting in the MBF and MCT.

Using the APWP, one can then generate the latitude history of Dingri as it moved together with stable India before the collision (Fig. 6). When this curve intersects the palaeolatitude for the B remagnetization, the age of this remagnetization is obtained: we interpret this age as the end of folding of the Dingri series, related to blocking of the Zangbo suture due to continental crust subduction. We find this age to be 50 Myr (that of magnetic anomalies 22–23). When error bounds are included for the palaeolatitudes of both magnetizations A and B and the

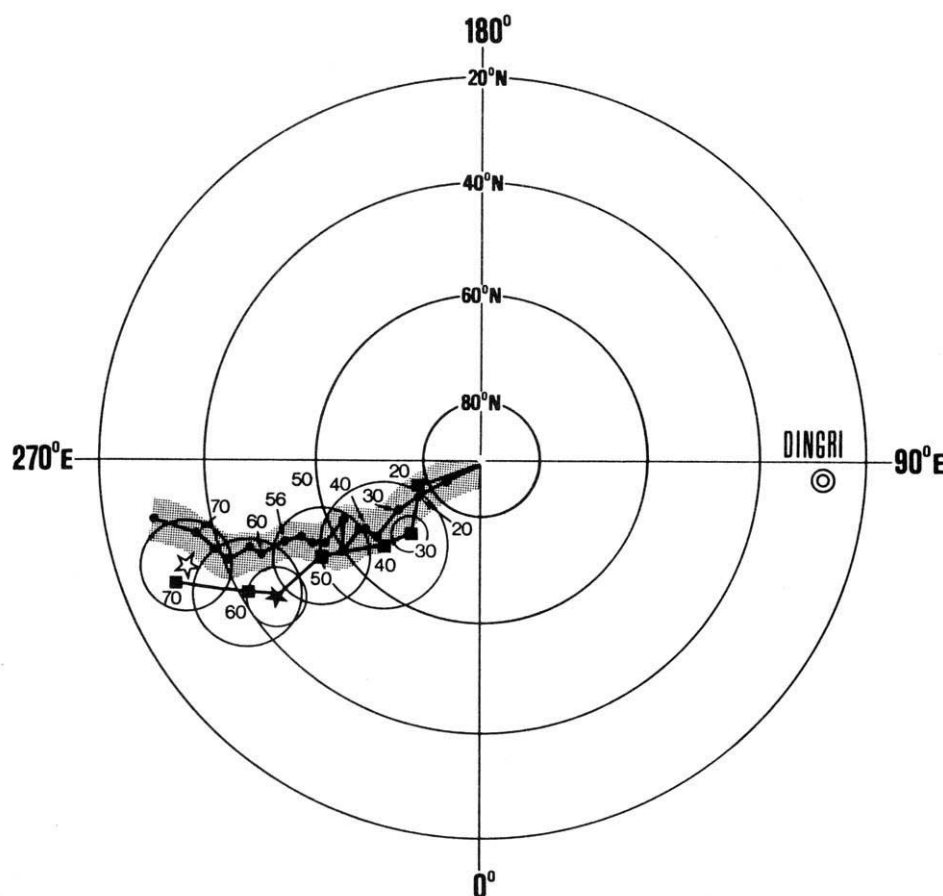


Fig. 5 Two estimates of the APWP for India. ■, From DSDP cores²⁰; ●, based on Indian Ocean magnetic anomalies (see refs 21, 23, text, and Table 2); ★, A DSDP pole for 57 Myr, recomputed from ref. 20 (see text); ☆, a 70 Myr Deccan traps pole²⁰.

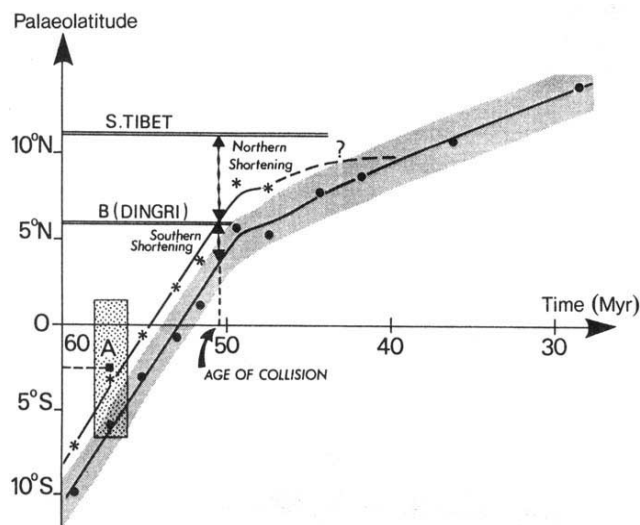


Fig. 6 The northward drift of a point now at Dingri following the motion of cratonic India, as deduced from the data of Fig. 5 and Table 2, is shown as a thick line with error estimates shaded. The primary (A) magnetization for Dingri, also shown with a shaded error box, provides an estimate for crustal shortening in the MBF and MCT to the south. The northward drift of Dingri (thick curve through A parallel to the Indian motion) intersects the B remagnetization, giving the age of the collision and, by comparison with data from southern Tibet⁴, the amount of crustal shortening in the Zangbo suture. Error estimates for those are omitted for clarity but are given in the text.

kinematic history of India, this age is found to range between 36 and 59 Myr. However, an age older than 57 Myr (deposition at Dingri) is geologically impossible. An age as young as 36 Myr implies the unlikely combination of (2σ) error bars, with the palaeolatitude of the B component at 10° N and the amount of convergence in the MBF and MCT reduced strictly to zero. The most probable age is unlikely to be older than 53 Myr or younger than 47 Myr (Fig. 6). The age of anomaly 22 happens to be that at which the relative and absolute motions given by Patriat and Achache²³ suddenly slow down and exhibit an erratic azimuthal behaviour which these authors also associate with the collision. Finally, the palaeolatitude of Dingri at 50 Myr can be compared with that of southern Tibet as determined by Achache *et al.*⁴, thus yielding an estimate of convergence in the Kangmar thrust

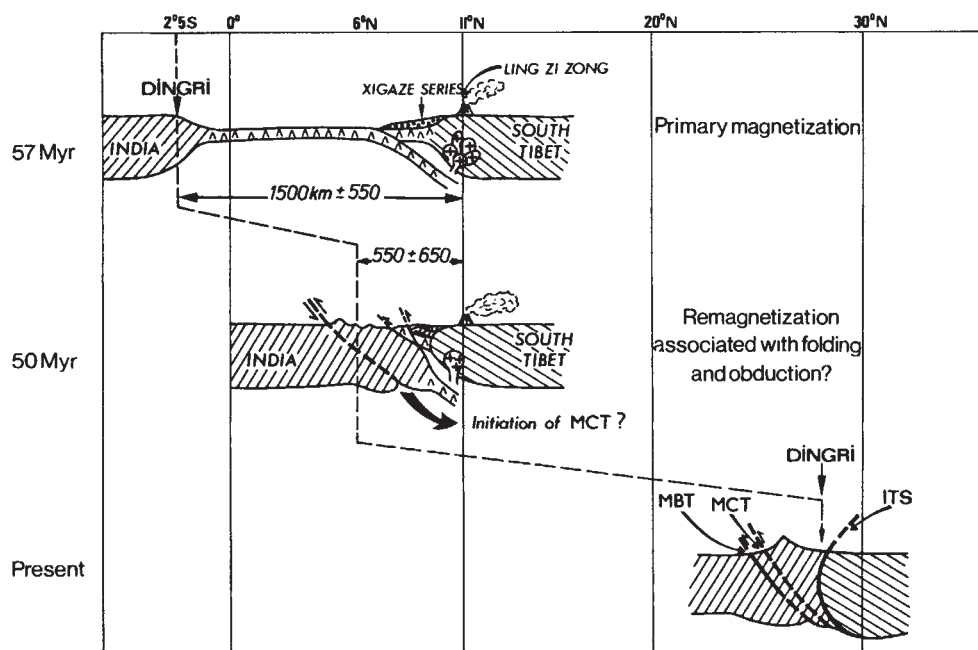
and Zangbo suture: $5^\circ \pm 6^\circ$. The uncertainty on this estimate is still rather large, and further age determinations from the Tibetan (60–40 Myr) volcanics will be helpful. Preliminary results from the ophiolitic complex and flysch series near Xigaze on the Zangbo suture²⁶ seem to indicate a remagnetization close to $6^\circ \text{ N} \pm 8^\circ$ which might have been acquired at the same time and under the same circumstances as remagnetization B at Dingri. This might indicate that the 5° estimate of convergence in the Zangbo is on the high side, but further work is needed to refine this analysis. Figure 6 can be complemented by tracing back the motion of the northernmost continental boundary of India before the collision. A schematic series of sections (Fig. 7) also displays the sequence of events before and immediately after the collision. The northern margin of India passed the Equator in the vicinity of Dingri around 56 Myr ago and emergence of Dingri occurred around 55 Myr. The collision (in the sense defined above) occurred around 50 Myr. The deformation history that followed may be deduced from the values presented here and previously published tectonic scenarios^{1–4,23}. Approximately 500 km of continental crust (or less) was subducted in the Zangbo before the suture was choked. Part of this may correspond to folding of the Dingri and Xigaze^{3,13} series and thrusting (around 40–20 Myr?) on the Kangmar thrust, and the estimate of subducted continental lithosphere might be reduced to a classical value around 300 km (refs 23, 27). When the suture became blocked (around 45 Myr? see refs 1, 2, 23), motion was transferred to the MCT and then MBF, much of the 400 km of convergence determined above occurring by thrusting between 45 and 35 Myr (refs. 28, 29). The amount of folding should, however, be subtracted from these values. Also, thrusting still proceeds to the south of the MBT and elsewhere^{9,30}. Propagating extrusion tectonics may have started concurrently to the north of the suture and may account for over two-thirds of the total Cenozoic convergence between India and Asia^{1–4}.

We thank H. Feinberg for biostratigraphical age determinations and P. Patriat for providing Indian Ocean data. This work was carried out within the Cooperative Research Project of the Chinese Ministry of Geology, Chinese Academy of Sciences, French Centre National de la Recherche Scientifique and Institut National d'Astronomie et de Géophysique. This is IGP contribution no. 782.

Received 6 June; accepted 20 August 1984.

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Fig. 7 Geodynamic evolution of the India–South Tibet collision assuming that the mean palaeolatitudes are exactly correct (compare with Fig. 6).



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Molecular cloning and expression of cDNAs for the human interleukin-2 receptor

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We have purified the human T-cell growth factor (interleukin-2) receptor and have cloned, sequenced and expressed cDNAs corresponding to this receptor. We identify one gene, but two interleukin-2 receptor mRNAs which differ in their polyadenylation signals. We have isolated an additional cDNA that may correspond to an alternatively spliced mRNA that lacks a 216 base segment and appears to encode an altered membrane protein which cannot bind interleukin-2.

INTERLEUKIN-2 (IL-2 or T-cell growth factor) is a lymphokine synthesized and secreted by some T cells following activation with antigen or mitogen in the presence of monocyte derived interleukin-1¹. IL-2 permits long-term growth *in vitro* of T-cell populations serving helper and cytotoxic functions²⁻⁴. It also augments natural killer cell activity⁵, and is being evaluated for possible therapeutic effects in patients with the acquired immunodeficiency syndrome and select neoplasms. IL-2 has been biochemically characterized⁶, molecularly cloned⁷⁻⁹, and is known to be encoded by a single gene^{10,11} on human chromosome 4.

In order to exert its biological effects, IL-2 must interact with specific high-affinity membrane receptors¹². This interaction is critical to the evolution of a normal immune response. IL-2 receptors are not expressed in resting T cells, but they are expressed rapidly following activation with antigen or mitogen. Thus, in contrast to most other hormone-receptor systems, this is an example where both ligand and receptor must be induced. IL-2 receptors are not present in most human leukaemic T-cell lines, but are expressed uniformly in high numbers in human T-cell leukaemia/lymphoma virus-I (HTLV-I)^{13,14} infected T cells¹⁵, and it is possible that these receptors may be involved in the uncontrolled growth of these cells.

The IL-2 receptor has been identified as an $M_r = 55,000$ glycoprotein on normal activated peripheral blood T cells¹⁶⁻¹⁸. These receptors are composed of a peptide precursor of $M_r = 33,000$ (p33). This precursor is cotranslationally modified to a p35/p37 doublet by N-linked carbohydrate addition, and then modified in the Golgi apparatus to the $M_r = 55,000$ form. Mature receptors contain N-linked and O-linked carbohydrate and sialic acid, and are both sulphated and phosphorylated. The receptors on some, but not all HTLV-I infected T-cell lines, vary slightly in mobility on SDS polyacrylamide gels. For example, the receptor on HUT-102B2 cells appears 5,000 daltons smaller than the receptor on normal activated peripheral blood T cells^{17,19}. These receptors share the same sized p33 and p35/p37

precursor forms, and the basis for their size difference is the result of altered post-translational modifications, including the amount of sialic acid and sulphate added.

Elucidation of IL-2 receptor structure, function and regulation of expression will improve our understanding of the early events of T-cell activation, including the mechanisms of membrane signal transduction. The availability of cDNA probes to the IL-2 receptor also allows further study of the relationship of these receptors to the adult T-cell leukaemia.

Purification and cloning of receptor

Using anti-Tac, an anti-IL-2 receptor monoclonal antibody^{16,19,20}, we purified the IL-2 receptor on an immunoaffinity column (Fig. 1). The purified protein, but not control eluates, blocked over 98% of human IL-2 induced proliferation of a mouse cytotoxic T lymphocyte line, suggesting that it indeed possesses IL-2 binding activity (data not shown).

We next determined the N-terminal 29 amino acids of the IL-2 receptor by a combination of sequencing unlabelled purified receptor on a gas phase sequencer and receptor biosynthetically labelled with ³⁵S-methionine, ³⁵S-cysteine, ³H-leucine, ³H-proline, or ³H-aspartic acid on a modified Beckman spinning cup sequencer (Table 1).

An oligonucleotide probe of 17 nucleotides and 64-fold degeneracy homologous to the (-)cDNA strand was synthesized, corresponding to the sequence Cys-Asp-Asp-Pro-Pro (amino acids three to eight). The 17mer was end-labelled with ³²P-ATP and polynucleotide kinase, and hybridized to Northern blots of HUT-102B2 mRNA to confirm that the 17mer identified at least one mRNA species. In fact, we detected two mRNAs approximately 1,500 and 3,500 bases long (data not shown).

A cDNA library (see Fig. 2 legend) was constructed in λ gt10 from HUT-102B2 cell mRNA according to the protocol of T. St John, J. Rosen and H. Gershenfeld (unpublished data), except that size-fractionated double-stranded cDNA was purified

Fig. 1 Purification of IL-2 receptor protein. Anti-Tac and control UPC10 immunoaffinity columns were prepared by coupling purified anti-Tac and UPC10 monoclonal antibodies to cyanogen bromide-activated Sepharose (Pharmacia). Approximately 8×10^{10} HUT-102B2 cells were grown in 10% fetal bovine serum/RPMI medium, collected by ultrafiltration, washed twice in phosphate buffered saline and solubilized in 100 ml extraction buffer (10 mM Tris pH 7.4, 0.15 M NaCl, 100 $\mu\text{g ml}^{-1}$ phenylmethylsulphonyl fluoride (PMSF), 0.5% NP40) for 45 min on ice with periodic vortexing. An additional 100 ml of extraction buffer was then added and the cells solubilized for an additional 30 min on ice. Extracts were then centrifuged in a Beckman Ti60 rotor at 37,000 r.p.m. for 30 min at 4 °C. The top lipid layer was discarded and the supernatant collected and stored in aliquots in liquid nitrogen. For each protein preparation, 10 ml aliquots of the extracted cells were passed over a UPC10 column four times, and then over an anti-Tac column three times. The anti-Tac column was washed with 40 ml extraction buffer, 40 ml of 10 mM Tris pH 7.5, 0.5 M NaCl, 0.5% NP40, 25 ml of 10 mM Tris pH 7.5, 0.1% NP40, and then 10 ml of 10 mM Tris pH 7.5. The anti-Tac column was eluted with 3 ml of 2.5% (v/v) acetic acid. The eluted protein was lyophilized, resuspended in 100 μl water and 5 μl analysed on SDS-polyacrylamide gels under reducing conditions: gels were silver stained to assess yield and purity³⁸. The columns were then washed extensively and stored in extraction buffer.

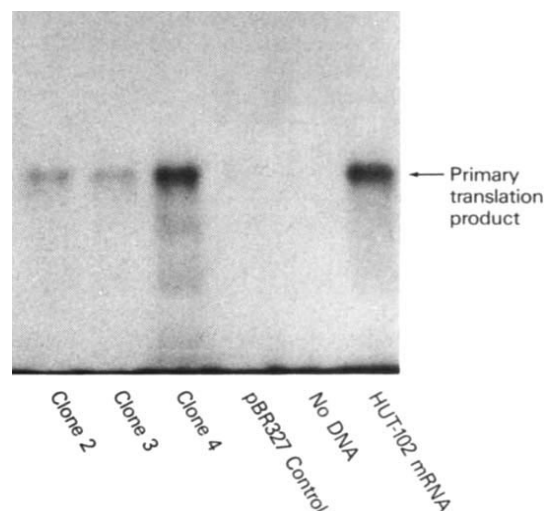
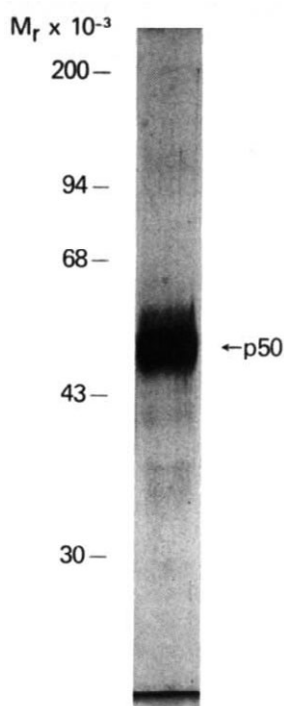


Fig. 2 Preparation of a rabbit heteroantiserum, identification of the primary translation product for the IL-2 receptor, screening of the cDNA library and selective hybridization. Purified IL-2 receptor protein (approximately 3–5 μg) was divided into equal aliquots, one boiled in 1% SDS for 5 min and one not treated, each emulsified with an equal volume of complete Freund's adjuvant, and injected subcutaneously into multiple sites in a New Zealand white female rabbit. The rabbit was boosted (analogous injections in complete Freund's adjuvant) at six weeks. The rabbit was phlebotomized weekly and the ability of the serum to block ^3H -anti-Tac binding to activated T cells and to precipitate the IL-2 receptor was assessed. The antiserum specifically precipitated the same 50,000 M_r band from HUT-102B2 cells as did anti-Tac (data not shown) and did not appear to specifically identify any other proteins. Total cellular RNA was isolated from HUT-102B2 cells by the method of Chirgwin *et al.*³⁹. The poly(A) fractions were isolated on oligo (dT)-cellulose (Collaborative Research) and translated for 2 h at room temperature in a wheat germ cell-free translation system as described by Gordon *et al.*⁴⁰. The translation mix was then boiled in 2% SDS by the method of Mostov *et al.*⁴¹, cooled to room temperature, diluted to five times the original volume with final concentrations of 1 $\times$ phosphate-buffered saline, 1% Triton X100 and 100 $\mu\text{g ml}^{-1}$ PMSF. The mix was cleared with normal rabbit serum and Cowan I strain staphylococcus⁴², immunoprecipitated with the heteroantiserum or preimmune serum as previously described and electrophoresed on a gel (sixth lane)¹⁹. The cDNA library consisted of 2.4 million recombinant phage. 500,000 phage from the library were amplified and approximately 200,000 recombinant phage from the amplified library were screened with the ^{32}P -kinased 17mer. Phage plaques were bound to nitrocellulose as described by Benton and Davis⁴³, prehybridized overnight at 50 °C in 6 $\times$ NET (1 $\times$ NET is 0.15 M NaCl, 0.001 M EDTA, 0.015 M Tris, pH 7.5), 5 $\times$ Denhardt's, 0.5% NP40, 200 $\mu\text{g ml}^{-1}$ salmon sperm DNA and then hybridized with the 17mer for 24–48 h in the same buffer containing 10% dextran sulphate. Filters were washed four times for 5 min in 6 $\times$ SSC on ice, then for 1 min at 50 °C, and then transferred immediately to 6 $\times$ SSC on ice, blotted dry and autoradiographed. Selective hybridization was performed²² with cDNA inserts from three phage clones subcloned into pBR322: pIL2R2, pIL2R3, and pIL2R4, and with pBR327 and omission of DNA as controls. DNA was linearized by digestion with *Sal*I prior to binding to nitrocellulose, HUT-102B2 mRNA was selectively hybridized, translated, and precipitated with the anti-IL-2 receptor heteroantiserum (first five lanes).

Table 1 Sequence of IL-2 receptor

H ₂ N-Glu-Leu-Cys-Asp-Asp-Asp-Pro-Pro-Glu-
Ile-Pro-His-Ala-Thr-Phe-Lys-Ala-Met-Ala-
Tyr-Lys-Glu-Gly-Thr-Met-Leu-Asn-Cys-Glu

Human IL-2 receptor from HUT-102B2 cells (purified using the anti-Tac affinity column) was subjected to automated Edman degradation on a gas phase sequencer and samples analysed by HPLC. Some positions were determined or confirmed by sequencing receptor biosynthetically labelled with ^3H -aspartic acid, ^3H -leucine or ^3H -proline, or with ^{35}S -methionine or ^{35}S -cysteine. For incorporation of radioactive amino acids, cells were grown in the presence of amino acid free RPMI, 5% fetal bovine serum (as a source of unlabelled amino acids) and 1–5 mCi of the radiolabelled amino acid. For aspartic acid, labelling was performed in the presence of 1 mM amino oxyacetic acid, as suggested by Coligan *et al.*³⁷. All radioactive amino acids used were the highest specific activity available from Amersham and/or New England Nuclear. *, The positions determined or confirmed by radiolabelled sequencing. The positions italicized represent the amino acids on which the oligonucleotide DNA sequence was based. The 17mer was synthesized and provided to us by Drs Rama M. Belagaje and J. Paul Burnett of Lilly Research Laboratories.

on glass silica mesh²¹ rather than by electroelution. In HUT-102B2 cells, IL-2 receptors account for approximately 0.05% of total cellular *de novo* protein synthesis¹⁶.

Eleven clones with inserts of 900–2,300 bases were identified by the 17mer as true positives. Inserts from three of the phage clones were subcloned into the *Eco*RI site of pBR322 (clones denoted pIL2R2, pIL2R3, and pIL2R4, with 900, 2,300, and 1,600 bases, respectively) to facilitate large-scale preparation of inserts. We performed selective hybridization experiments by

the method of Maniatis *et al.*²². All three recombinant plasmids hybridize mRNA that, when translated in a wheat germ cell-free translation system and immunoprecipitated with a rabbit heteroantiserum to the IL-2 receptor, yield the same sized primary translation product precipitated directly from translations with HUT-102B2 mRNA (Fig. 2). In contrast, control pBR327 and nitrocellulose without DNA added does not selectively hybridize to the IL-2 receptor mRNA. These data confirm that clones 2, 3 and 4 are receptor-associated clones.

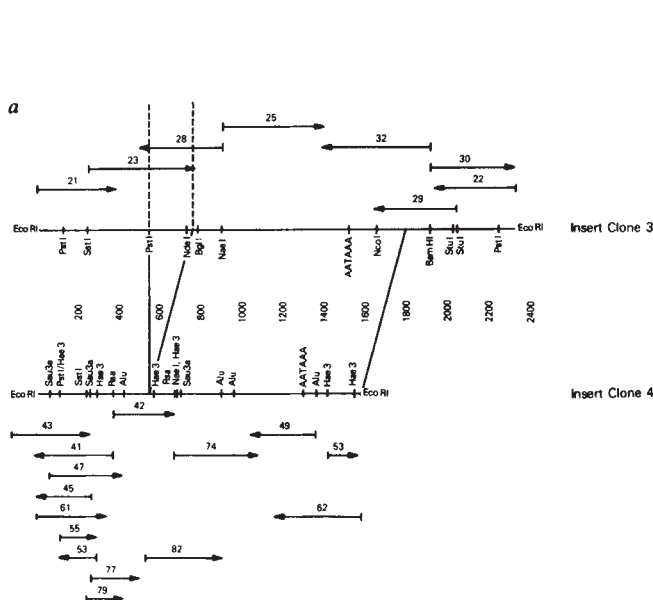


Fig. 3 Restriction map, sequencing strategy, DNA sequence and corresponding protein sequence of IL-2 receptor cDNA. *a*, Restriction map of cDNA inserts of pIL2R3 (clone 3) and pIL2R4 (clone 4). Note the location (vertical dashed lines) of the splicable segment in pIL2R3 that is not present in pIL2R4. Clone 3 was directionally cloned into M13 phage using *EcoRI*, *SstI*, *NaeI*, *BamHI*, and *StuI* restriction sites. Clone 4 was sequenced using four base restriction enzymes *RsaI*, *HaeIII*, *AluI*, and *Sau3aI*. *b*, DNA and protein sequences for the IL-2 receptor. The DNA sequences of clones 3 (top row) and 4 (second row) are shown with deduced amino acid sequence (third row). The N-terminal amino acid (glutamic acid) and the AATAAA polyadenylation signal which may be used in the 1,500 base mRNA are underlined. The T at 895 (under the asterisk) in one of four sequencing runs was a C. We cannot distinguish with certainty whether position 1,604 (under the asterisk) is a C or a T, which may represent a difference between the two clones.

Sequence of pIL2R3 and pIL2R4 cDNA inserts

The inserts of pIL2R2, pIL2R3 and pIL2R4 were subcloned into M13 bacteriophage and their complete nucleotide sequences by the dideoxy chain termination method²³⁻²⁵. The restriction maps and sequencing strategy of pIL2R3 and pIL2R4 inserts (Fig. 3a) and the DNA sequences for each (Fig. 3b) are shown. We analysed and compared the DNA sequences on an IBM system 370 computer using the program described by Queen and Korn²⁷ and with the assistance of Dr M. Kanehisa on a Vax computer (Digital Equipment Corporation). Clones 2, 3 and 4 each contain long open reading frames, including the 87 bases corresponding to the 29 amino acids determined by protein sequencing. The N-terminal amino acid of the mature receptor is located at base 244. There are three potential initiator ATGs in the correct reading frame at amino acid positions -21, -15, and -7 (bases 181-183, 199-201, and 223-225, respectively). Preliminary data from sequencing of an ³⁵S-methionine labelled primary translation product suggest that the initiation site is at amino acid -21. All clones had C-tails at the 5' end added during cDNA synthesis (St John, Rosen and Gershensfeld, unpublished data), but lacked 3' poly(A) tails. We hypothesize that the poly(A) tails and a part of the 3' untranslated region

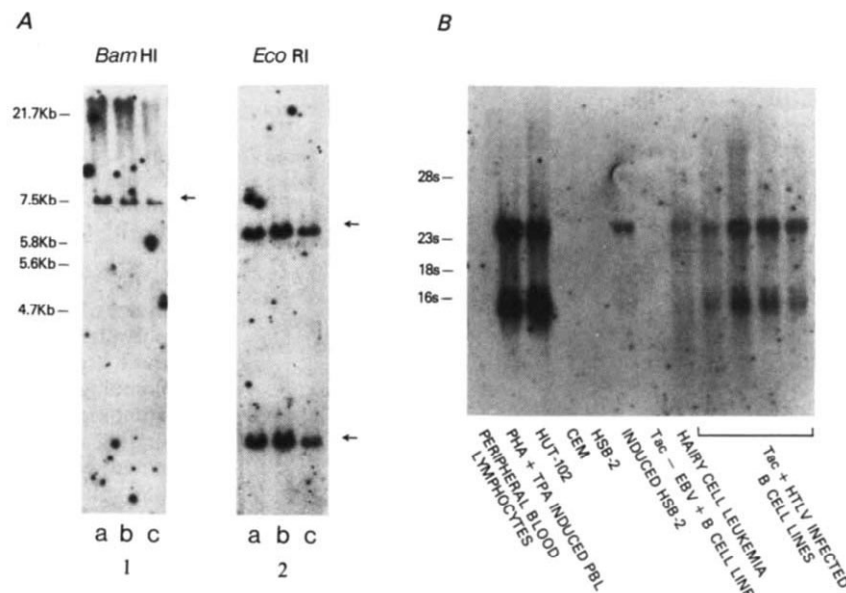
may have been lost due to 5' to 3' exonuclease activity on the first strand cDNA during second-strand synthesis, since the holoenzyme DNA polymerase I instead of the Klenow fragment is used in the protocol.

Analysis of the sequence of clones 3 and 4 reveals that they differ in two principal ways. First, clone 4 encodes a smaller peptide (179 amino acids; 20,070 daltons) than does clone 3 (251 amino acids; 28,437 daltons). These proteins share the same amino- (base 244) and carboxy- termini (TAG at 997-999 in clone 3) and both contain 2 potential N-linked glycosylation sites (asparagines at bases 388-390 and 445-447), but clone 4 lacks 216 bases (72 amino acids) in the middle of the protein coding region. In clone 3, this region is present and is bounded by the sequence TTCCAGGT on both sides (bases 542-549 and 758-765), and therefore is a segment flanked by a typical messenger RNA donor and acceptor splice site^{27,28}. Second, clone 3 extends further 3' than clone 4, but neither clone reaches a terminal polyadenylation signal sequence (AATAAA).

Expression of IL-2 receptor cDNA

We predict a molecular mass for the IL-2 receptor of 33,000 daltons based on apparent *M_r* on SDS gels for the putative

Fig. 4 **A**, The IL-2 receptor appears to be encoded by a single-copy gene. DNA from human placenta (lane *a* of each panel) and tonsils (lanes *b* and *c* of each panel) was digested with *Bam*HI and *Eco*RI, transferred to nitrocellulose, and hybridized with nick-translated pIL2R2. The blotted DNA was provided by Dr Ajay Bakhshi. DNA was digested with *Bam*HI (panel 1) and with *Eco*RI (panel 2). **B**, IL-2 receptor mRNA expression in various lymphoid cell types. 10 µg of poly(A)⁺ RNA from each indicated cell type was electrophoresed on a formaldehyde gel, transferred to nitrocellulose and hybridized with nick-translated pIL2R3.



peptide precursor. It seems probable that clone 3, which encodes the larger protein product, is the correct one, despite the variability that can exist in migration of different proteins on SDS gels due to variable association with SDS up to the theoretical level of 1.4 g SDS per gram of protein. To test this hypothesis, we expressed each of the cDNAs in COS-1 cells using the expression vector pcEXV-1, which places the cDNA under the control of the Simian virus 40 (SV40) early promoter and SV40 enhancer sequences (Table 2). Cells transfected with control pBR322 do not bind either ³H-IL-2 or ³H-anti-Tac, whereas cells transfected with the insert from pIL2R3 bind both ³H-IL-2 and ³H-anti-Tac. This binding is specific, as it was completely blocked by excess unlabelled IL-2 and anti-Tac. The receptor on these cells has an apparent *M_r* of 50,000, apparently identical to the receptor on HUT-102B2 cells, and smaller than the receptor on PHA activated T cells (*M_r* of 55,000). Transfection with pcEXV-1 containing the clone 4 insert, which encodes a smaller protein than clone 3, does not result in the expression of IL-2 receptors (Table 2). As both expression vector constructs were active transcriptionally, these data demonstrate that clone 3 encodes a functional IL-2 receptor and that clone 4 does not.

A single gene encodes IL-2 receptor

We next evaluated the number of genes corresponding to the IL-2 receptor by hybridizing nick-translated ³²P-pIL2R2 to genomic DNA digested with *Eco*RI and *Bam*HI. pIL2R2 has

an insert corresponding to bases 1-937 and therefore lacks the C-terminal region of the protein and the 3' untranslated region. We identified two fragments in the *Eco*RI digests, the larger of which is 5,800 bases long with a third faint band of 10,000 bases (Fig. 4A). In the *Bam*HI digests, we identified a major 7,500-base fragment and a second less intense more diffuse band of over 25 kilobases (kb). Hybridization with full-length IL-2 receptor cDNA reveals *Eco*RI genomic fragments of 10, 5.8, 2.4 kb and two smaller fragments, and *Bam*HI fragments of 25 and 7.5 kb. These data suggest a single copy gene, but do not exclude a recently duplicated gene.

mRNA expression and HTLV-I infection

Using an IL-2 receptor cDNA, we find that the pattern of expression of IL-2 receptor mRNA parallels the expression of Tac antigen. Resting Tac-negative peripheral blood T cells contain few, if any, IL-2 receptor mRNA copies (Fig. 4B, lane 1). However, Tac-positive peripheral blood T cells activated with phytohaemagglutinin (PHA) plus phorbol myristate acetate (PMA) (Fig. 4B, lane 2), and HTLV-1-infected HUT-102B2 cells (Fig. 4B, lane 3), contain two mRNAs (approximately 1,500 and 3,500 bases), which hybridize to nick-translated IL-2 receptor cDNA. Tac-negative CEM and HSB-2 acute lymphocytic leukaemic T-cell lines do not contain IL-2 receptor mRNA; induction of Tac expression on HSB-2 cells with PMA²⁹, however, is associated with the appearance of IL-2 receptor

Table 2 Expression of IL-2 receptors in COS-1 cells

Transfected plasmid	Experiment 1		Experiment 2	
	Unlabelled IL-2 added	³ H-IL-2 d.p.m. bound	Unlabelled anti-Tac added	³ H-anti-Tac d.p.m. bound
pBR322	—	25	—	60
pBR322	+	17	+	150
pcEXV-1-IL2R3	—	562 ± 11	—	28,588 ± 1,548
pcEXV-1-IL2R3	+	62 ± 18	+	261 ± 0
pcEXV-1-IL2R4	—	12 ± 10	—	252 ± 9
pcEXV-1-IL2R4	+	12 ± 3	+	171 ± 39

0.5 µg of clone 3 or 4 insert were ligated to 0.25 µg of *Eco*RI digested and alkaline phosphatase treated pcEXV-1 (provided by Drs J. Miller and R. Germain). The plasmid was transformed into competent cells and recombinant plasmids with the inserts in the correct orientations selected. 5 µg of each recombinant plasmid or of control pBR322 were transfected into COS-1 cells using calcium-phosphate precipitation. The cells were grown overnight, media changed, and then grown for 48 h. The cells were washed, incubated for 90 min at 4 °C with RPMI 1640 medium containing 3% fetal bovine serum and tested for expression of IL-2 receptors. In experiment 1, 45,000 d.p.m. of ³H-IL-2 with or without a 500-fold excess of unlabelled IL-2 were added. In experiment 2, 194,000 d.p.m. of ³H-anti-Tac with or without a 1,000-fold excess of anti-Tac were added. The cells were then washed four times in medium containing fetal bovine serum and dissolved in 1 ml 0.1 M NaOH. The cell-associated radioactivity was then determined. The machine background of 44 d.p.m. has been subtracted from each data point. Data are expressed as mean d.p.m. bound ± standard deviation determined from duplicate plates. For reference, HUT-102B2 cells, which have approximately 300,000 total (high plus low affinity) IL-2 receptors per cell, bound either 1,160 of 6,500 d.p.m. of ³H-IL-2 or 34,500 of 92,100 d.p.m. of ³H-anti-Tac added per 10⁶ HUT-102B2 cells.

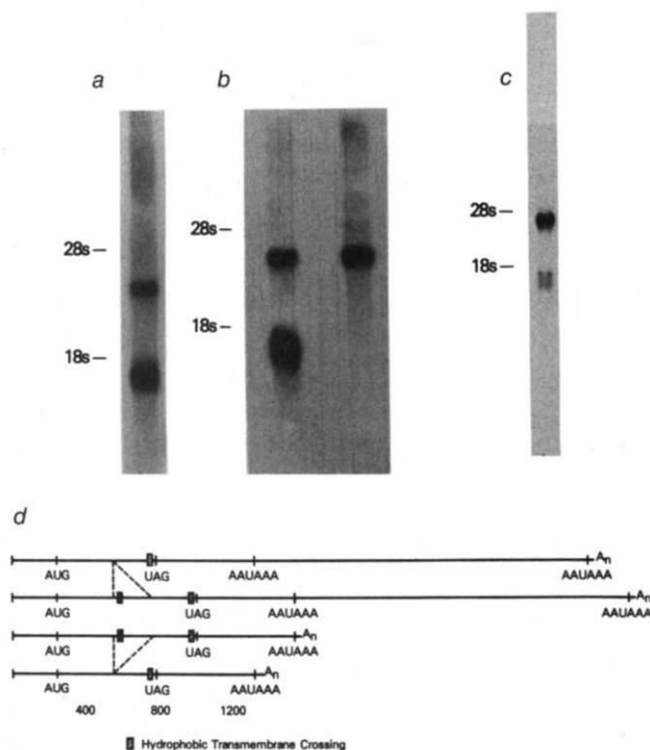


Fig. 5 *a*, Hybridization to HUT-102B2 mRNA with 32 P-labelled *Pst*I-*Nde*I segment from pIL2R3. Washes were performed in $0.1 \times$ SSC, 0.1% SDS at 52 °C. *b*, Hybridization to HUT-102B2 mRNA with 32 P-labelled pIL2R3 (left lane) or *Nco*I-*Eco*RI 3' segment of pIL2R3. *c*, Hybridization of 32 P-labelled pIL2R3 to mRNA from PHA activated normal T cells. Note that the smaller mRNA appears to be a doublet. *d*, Four possible mRNAs from the IL-2 receptor gene. The top two mRNA (3,300 and 3,500 bases, corresponding to clones 4 and 3, respectively) utilize the same AUAUAA but differ in that the 3,300-base mRNA splices out and the 3,500-base mRNA retains a 216-base segment. The third and fourth are like the second and first, respectively, except that they utilize a more proximal AUAUAA. The third mRNA represents the 1,500-base mRNA retaining the region and the fourth has lost the region and is similar to the 3,300-base mRNA (doublet in *c*).

mRNA. The induced HSB-2 cells express the two mRNAs in significantly different relative quantities. A Tac-negative Epstein-Barr virus (EBV)-transformed B-cell line is negative, but Tac-positive hairy-cell leukaemia cells³⁰ and Tac-positive HTLV-infected EBV negative B-cell lines (provided by Drs D. Longo and D. Mann) express both IL-2 receptor mRNAs. These data provide a pattern of mRNA expression consistent with our knowledge of which cells react with anti-Tac and express IL-2 receptors, and demonstrate that even in B cells, HTLV-I infection is associated with IL-2 receptor mRNA expression.

We next attempted to determine how the 1,500- and 3,500-base mRNAs differ. A *Pst*I/*Nde*I segment probe from clone 3 (bases 552-729), which contains most of the 216-base pair segment spliced from clone 3 to generate clone 4, hybridizes to both the 1,500- and 3,500-base mRNAs (Fig. 5a). These data suggest that both mRNAs contain the segment and that neither corresponds to clone 4. From the sequence and expression data, which together indicate that nonsplicing of the 216-base pair region correlates with IL-2 receptor expression, we hypothesize that both the 1,500- and 3,500-base mRNAs are capable of encoding functional IL-2 receptors. In support of our hypothesis, when mRNA is size-fractionated on a methyl mercuric hydroxide gel, the gel sliced, and RNA contained in the slices translated and immunoprecipitated, the correct primary translation product is identified from either the 1,500- or 3,500-base mRNAs (data not shown).

Both clones 3 and 4 contain two AATAAA sequences corresponding to the potential polyadenylation addition signal pres-

ent in eukaryotic mRNAs (the first is in the coding region, bases 351-356, and the second is 3' to the coding region, beginning at base 1,523 in clone 3, and is underlined in Fig. 3b). In addition, there is an AATAAA-like sequence (ATTAAA) at bases 1,298-1,303. These AATAAAs apparently are not utilized in clones 3 and 4, as no poly(A) tails are noted and the cDNAs continue 3' to the AATAAA sites. The functional AATAAAs in clones 3 and 4 are presumably 3' to the ends of our clones. The *Nco*I-*Eco*RI (Fig. 5b) and *Bam*HI-*Eco*RI 3' fragments of clone 3 (bases 1,642-2,335 and 1,921-2,335, respectively) hybridize only to the 3,500-base mRNA. Thus, we propose that the difference in size of the IL-2 receptor mRNAs is the result of using different polyadenylation signal sequences. In the *Bam*HI-*Eco*RI region, there are two sets of repeated sequences (2,080-2,113 is 82% homologous to 2,213-2,247, and 2,115-2,137 is 87% homologous to 2,279-2,301), although the significance of this finding is unclear. Clone 4 does not arise from either message noted, but its mRNA is identical to that of clone 3 except that the splicable segment has been removed. Such an mRNA (approximately 3,300 bases) would not be resolved from the 3,500-base mRNA corresponding to clone 3 on the Northern blot.

Conclusions

The IL-2 receptor sequence described here has been compared with the Biomedical Research Foundation and Los Alamos databases; no significant homology was found to other known proteins or DNA sequences. Lack of homology to HTLV-I excludes the possibility that this virus encodes the IL-2 receptor. We have shown previously that the primary translation product and other precursor forms of the IL-2 receptor expressed on HUT-102B2 cells are identical in size to those of PHA lymphoblasts, but that there are differences in post-translational processing¹⁷. Point mutations may thus exist which distinguish these two receptors.

Sequencing of the cDNAs suggests the possibility of alternate mRNA splicing, wherein a 216 base, 72 amino acids long, internal region of the protein may be excised. Whether this region is retained or removed, the encoded protein uses the same initiation and termination sites and shares the identical protein sequence except in the region of splicing. Alternate mRNA splicing has been implicated in the control of gene expression in a variety of different cellular systems. For example, the splicing of alternative exons results in the synthesis of membrane versus secreted forms of immunoglobulin δ and μ genes at different stages in B-lymphocyte maturation^{31,32}. Similarly, transcripts from the calcitonin gene are alternatively spliced in neural tissue to produce a calcitonin gene-related peptide which is distinct from the product found in thyroid tissue³³. Transcripts from the α -amylase and *c-myc* genes in different tissues are differentially processed without altering the protein product³⁴. The rat and human γ -fibrinogen genes can be spliced alternatively in the same cell to produce proteins with different functions³⁵. Splicing of a segment within the coding region of the IL-2 receptor mRNA appears to change the function of the resultant protein. Transfection of the unspliced cDNA clone in COS-1 cells results in the expression of membrane receptors capable of binding both purified radiolabelled IL-2 and anti-Tac. Transfection of the spliced form of the cDNA, however, does not result in the expression of IL-2 receptors, even though specific mRNA is detected in the transfected cells (data not shown). We assume that this mRNA is translated into protein product that does not bind IL-2, but thus far we have not been able to precipitate the 'spliced' protein product from either surface iodinated or 35 S-methionine biosynthetically labelled COS-1 cells. It is also possible that the mRNA is unstable, ineffectively translated, or that the resulting protein product is rapidly degraded. Nevertheless, these data suggest that IL-2 receptor expression may not be regulated solely at the level of transcription, but rather also through post-transcriptional changes in mRNA processing. We cannot exclude the possibility that clone 4 represents a cloning artefact, but the presence of typical mRNA splice signals makes this less likely.

We hypothesize that the four different mRNAs may exist in two pairs, with one of each pair retaining and one lacking the splicable region (Fig. 5d). The doublet in the 1,500-base mRNA (Fig. 5c) is consistent with the presence of a spliced mRNA, but could also be due to utilization of the ATTAAA sequence.

The DNA sequence of clone 3 permitted us to deduce the entire protein sequence of the human IL-2 receptor. The carboxy-terminus of the protein encoded by these mRNAs contains a stretch of positively charged basic amino acids (6 of the terminal 13 amino acids are arginines or lysines), which we presume to be intracytoplasmic. This region contains one serine and one threonine, which may serve as potential phosphorylation sites¹⁷. This region is preceded by a hydrophobic region 19 amino acids long which could readily form a transmembrane region (Fig. 5d). Both these regions are typical for membrane proteins³⁶. There is a second hydrophobic region which is included in the splicable region. Although it is conceivable that this region represents a second transmembrane crossing, the presence of amides (histidine and glutamines) makes this unlikely. If this were a second transmembrane crossing, the two N-linked glycosylation sites would be intracytoplasmic, which is without precedent. It is possible that this region may represent

a fold in the protein, a loop into but not through the membrane, or may be important in binding IL-2, which itself has hydrophobic regions. Presumably both of the potential N-linked glycosylation sites in the protein are used, as we have identified previously two discrete N-linked precursor forms with apparent M_s of 35,000 and 37,000¹⁶. In addition, there are numerous serine and threonines immediately extracytoplasmic to the transmembrane region which may serve as the potential O-linked glycosylation sites which exist in this protein¹⁶. The mechanism of signal transduction by this receptor remains unclear, especially in view of the very short cytoplasmic tail (13 amino acids long) which would appear too short to mediate an enzymatic activity. Whether the ligand itself communicates the signal to the cell or whether other proteins serve as subunits in a receptor complex, as has been suggested^{16,19}, is under further investigation.

Note added in proof: Preliminary S_1 nuclease protection assays suggest that HUT-102B2 cells contain both the spliced and unspliced forms of mRNA indicated in Fig. 5d. Since submission of this paper, Wano *et al.*⁴⁴ have characterized IL-2 receptors on HUT-102B2 cells and PHA lymphoblasts, with findings similar to those in refs 16-19.

Received 1 June; accepted 17 August 1984.

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Molecular cloning of cDNA encoding human interleukin-2 receptor

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The human interleukin-2 (IL-2) receptor was purified by affinity chromatography using the anti-Tac monoclonal antibody, and its N-terminal amino acid sequence was determined. Complementary DNA clones were isolated and sequenced to reveal the primary structure of the IL-2 receptor precursor, which has 272 amino acid residues. The receptor is separated into two domains by a putative 19-residue transmembrane region. Two mRNAs (1.4 and 3.5 kilobases) hybridizing to the cDNA clone were found in human T cells bearing the IL-2 receptor. The cDNA directed synthesis of the IL-2 receptor in COS cells.

THE physiological proliferation of T lymphocytes (T cells) requires a specific interaction between a hormone-like peptide, T-cell growth factor (TCGF)¹ or interleukin-2 (IL-2)², and its cell-surface receptor³⁻⁶. Human IL-2, a peptide of ~14,000 molecular weight (M_r)⁷, produced by activated T cells, allows

the continuous *in vitro* proliferation of T cells bearing the IL-2 receptor. The immunological activation of G_0 -phase T cells, which fail to receive the growth signal by the ligand, requires not only the presence of IL-2 but also expression of the IL-2 receptor. Experiments from several laboratories^{8,9} have

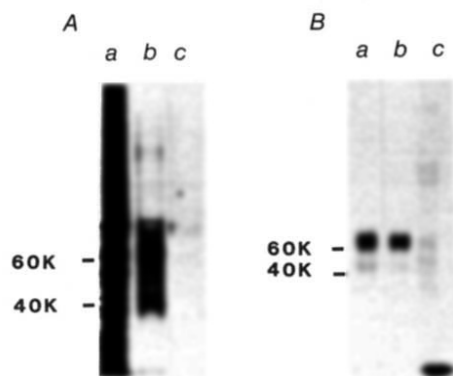


Fig. 1 Purification of the Tac antigen and IL-2 receptor. **A**, SDS-polyacrylamide gel electrophoresis of purified Tac antigen. *a*, Effluent fraction of anti-Tac-Sepharose column (20 µg); *b*, eluted fraction of anti-Tac-Sepharose column (0.1 µg); *c*, buffer alone (to show staining background). **B**, SDS-polyacrylamide gel electrophoresis of the IL-2 receptor of MT-1 cells surface-labelled with ^{125}I -NaI. *a*, Eluate from IL-2-Affigel-10 column (4,250 c.p.m.); *b*, eluate from anti-Tac-Sepharose column (5,200 c.p.m.); *c*, total extracts (4,800 c.p.m.).

Methods: **A**, MT-1 cells (5×10^9 cells) were solubilized with buffer (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 1% NP40, 1 mM phenyl methyl sulphonyl fluoride (PMSF)). The lysates were absorbed successively with bovine serum albumin-conjugated Sepharose 4B and immunoglobulin (human and mouse)-conjugated Sepharose 4B. The effluent fractions of the solubilized materials were applied to a column (0.5 × 2 cm) of the anti-Tac antibody coupled with Sepharose 4B (5 mg protein ml $^{-1}$ beads). After extensive washing of the column with PMSF-free solubilizing buffer and a high-salt buffer (10 mM Tris-HCl, pH 7.4, 1% NP40, 1 M NaCl), the Tac antigen was eluted with 0.2 M sodium citrate buffer, pH 2.6, containing 1% NP40. The eluate was neutralized with 2 M Tris and rechromatographed with an anti-Tac-Sepharose column. An aliquot of the eluted material was precipitated with 10% trichloroacetic acid and run on a 7.5% SDS-polyacrylamide gel⁶, analysed with silver stain. **B**, MT-1 cells were labelled with ^{125}I using lactoperoxidase⁶. The solubilized materials were purified by a column of anti-Tac-Sepharose or Affigel-10 conjugated with IL-2 synthesized by recombinant DNA in *Escherichia coli* (1.8 mg IL-2 per ml gel). Eluates from each column and the original extracts were run on a 7.5% SDS-polyacrylamide gel autoradiographed.

demonstrated that antigenic stimulation induces temporal expression of the IL-2 receptor on resting T cells as well as cloned T-cell lines, indicating that the antigen-mediated regulation of the IL-2 receptor expression has an essential role in the clonal expansion of the antigen-specific T cell. As most peripheral as well as thymic T cells do not carry the receptor *in vivo*, the regulatory expression of the IL-2 receptor appears to be a safeguard against a catastrophic spread of the T-cell proliferation by an immunogenic stimulus.

By contrast, an excessive number of IL-2 receptor molecules are expressed on leukaemic cells^{10,11} of adult T-cell leukaemia (ATL) endemic in parts of Japan and the Caribbean¹²⁻¹⁴. The leukaemic T cells and the T-cell lines established from these patients contain the DNA sequence of a unique C-type retrovirus, human T-cell leukaemia virus (HTLV)¹⁵ or adult T-cell leukaemia virus (ATLV)¹⁶. A monoclonal antibody (anti-Tac) produced by using ATL cells as antigens¹⁷, recognizes determinants associated with the IL-2 receptor^{18,19}. The number of IL-2 receptors of normal T cells is reduced by interaction with the anti-Tac antibody (down-regulation)²⁰. This down-regulation is another abnormal feature of IL-2 receptor expression of ATL cells¹¹. This aberrant and excessive expression of the IL-2 receptor might be related to leukaemogenic proliferation of ATL cells²¹⁻²³, thus the structure of the IL-2 receptor and regulation of its expression appear to be essential for elucidation of molecular mechanisms of ATL cells. Biochemical analyses of the IL-2 receptor is facilitated by the recent availability of purified IL-2 and monoclonal antibodies recognizing determinants associated with the human and rodent IL-2 recep-

Table 1 Expression of Tac-2 cDNA in COS-7 cells

DNA	Antibody	% Positive	MFI
pKCR·Tac-2·A	Expt 1 X5563	1.4	18.8
	Anti-Tac	22.9	56.3
	Expt 2 X5563	1.6	20.2
	Anti-Tac	16.8	46.5
pKCR·Tac-2	X5563	1.4	19.0
	Anti-Tac	7.3	30.4
pKCR·Tac-2·Ai	X5563	3.5	23.4
	Anti-Tac	3.4	24.3
pKCRH2	X5563	1.0	18.5
	Anti-Tac	3.1	24.9

COS-7 cells⁴⁶ (500,000) maintained in Dulbecco's minimal essential medium (DMEM) with 5% fetal calf serum (FCS) were seeded onto disposable tissue culture dishes. Medium was washed after 20 h. 4 h later, the cells were supplemented with 1/10th volume of transfection mixtures each containing 40 µg ml $^{-1}$ of the plasmid DNA indicated^{47,48}. After 12 h incubation, cells were exposed to 2.5% glycerol in DMEM at room temperature for 1 min, washed and further incubated in DMEM with 8% FCS for 48 h at 37 °C⁴⁵. The cells were collected and stained with anti-Tac monoclonal antibody and fluoresceinated goat anti-mouse antibody (Cappel) as described elsewhere¹⁷. As paired control experiments, a murine myeloma protein, X5563, replaced anti-Tac antibody. The expression of Tac antigen on the cells were determined by flow cytometry using Spectrum III (Ortho). MFI, mean fluorescence intensity.

tors^{8,9,24-26}. The receptor molecules in these species appear to be similar glycoproteins of about 55,000–60,000 *M* as assessed by mobility in SDS-polyacrylamide gels. Intracellular precursor molecules of the human receptor have smaller molecular sizes, of ~40,000 (refs 5, 6).

IL-2 receptor sequence

We purified the Tac antigen (the putative IL-2 receptor) from an ATL cell line MT-1 (ref. 27) by affinity chromatography with the anti-Tac monoclonal antibody. We find two components of MT-1 of 60,000–65,000 *M*_r and 40,000–45,000 *M*_r (Fig. 1A). The broad precipitated bands on our gels were essentially the same as those of material internally labelled with ^{35}S -methionine as described previously^{5,6}. Pulse-chase experiments have clearly demonstrated that the lower molecular component is a precursor to the surface receptor^{5,6}. We also obtained the identical two components by purification with Sepharose conjugated with IL-2 (Fig. 1B); we thus confirm here the previous conclusion that the Tac antigen is present on the IL-2 receptor molecule.

The molecular size difference of the two components is thought to be due to different extents of glycosylation; we therefore sought to determine the N-terminal amino acid sequence of the affinity-purified material containing the two components (Fig. 2A). We find a unique amino acid sequence from the mixture of the two components, which indicates the identity of the peptide moieties of the two components, although we cannot exclude the possibility that the N-terminus of one of the components is blocked. Short oligonucleotides (14 bases) corresponding to residues 4–8 (Asp-Asp-Asp-Pro-Pro) were synthesized as a probe for screening cDNA libraries.

cDNA clones

A cDNA library complementary to poly(A)⁺ mRNA of MT-1 cells was constructed and screened with the ^{32}P -labelled 14-base oligonucleotides. The synthetic probes hybridized with three out of 4×10^5 colonies of the MT-1 cDNA library. In the second screening, two of the three candidates hybridized with the probes. After isolation and propagation of the two colonies designated Tac-1 and Tac-2, we cut out the inserts by double digestion with *Hind*III and *Pvu*II; both inserts hybridize with the oligonucleotide probes. Restriction site mapping and nucleotide sequence determination show that the two clones are identical, except that the Tac-2 is 16 bases longer at its 5' end than Tac-1.

The complete nucleotide sequence (1,308 base pairs, bp) of the Tac-2 insert was determined according to the strategy shown

per cell whereas ATL-2 expresses about 8,000 molecules per cell. Our results unequivocally demonstrate that the Tac-2 sequence encodes the IL-2 receptor.

Discussion

The amino acid sequence of the IL-2 receptor does not have any significant homology with any known eukaryotic genes (or oncogenes). Cloning of the IL-2 receptor gene will, however, allow new insights into the physiological function and mechanism by which the IL-2 and its receptor system regulates T-cell proliferation. These include elucidation of the three-dimensional structures of the ligand and receptor which can be produced in large quantities in *E. coli* or mammalian cells, and the molecular mechanisms of signal transmission via surface receptor in the hormone and lymphokine systems, via mutants of the IL-2 receptor gene.

Cloning of the IL-2 receptor gene will also allow us to test several hypotheses proposed for the mechanism of leukaemogenesis of ATL triggered by ATL-2, which has no typical oncogene sequence³³. The excessive numbers of IL-2 receptor on ATL cells may be similar to aberrant expression of the EGF receptor on certain transformed cell lines such as A431

(ref. 34). The homology between the *erb-B* oncogene and the cytoplasmic domain of the EGF receptor²⁸ has strengthened the hypothesis²¹⁻²³ that an excessive amount of the IL-2 receptor might alter the normal growth control of T cells. This can be directly tested by transfection of human T-cell lines by the IL-2 receptor cDNA clone. We can also examine whether the IL-2 receptor gene in ATL cells has any mutations. A unique lymphokine secreted from ATL cells, ATL-derived factor (ADF), enhances the expression of the IL-2 receptor³⁰. ADF may be responsible for the continued expression of the IL-2 receptor in many ATL-2-positive T-cell lines. It is of interest to know whether ADF is able to induce transcription of the IL-2 receptor gene in human T cells.

We thank Japan Scientific Instrument Co. (JSIC) and Dr I. Kubota (Suntory Ltd) for amino acid sequence determination, JSIC for oligonucleotide synthesis, Dr K. Kato (Takeda Chemical Co.) for supply of IL-2 and Affi gel-conjugated IL-2, Dr T. Miyata for computer analysis of the sequence, and Dr M. Okada, Mr Y. Tagaya and Dr T. Noma for technical assistance. This investigation was supported by grants for special promotion of science from Ministry of Education, Science and Culture of Japan.

Received 28 June; accepted 17 July 1984.

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LETTERS TO NATURE

Terrestrial catastrophism—Nemesis or Galaxy?

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The ~30 Myr periodicity associated with the Sun's motion through the central plane has been linked to geomagnetic reversals^{1,2}, biological extinctions³ and crater ages⁴⁻⁶. This periodicity is consistent with galactic theories of terrestrial catastrophism^{1,5}. It has been suggested, however, that the periodicity is controlled by a hypothetical stellar companion of the Sun ('Nemesis') in a highly eccentric orbit of arbitrarily chosen period which periodically upsets the Oort cloud^{7,8}. Although the idea appears superficially attractive, there has been little or no attempt to relate it to what is already known about the Oort cloud and the environment in which the Sun-Nemesis system would have to exist. Thus, the inferred phase of the Nemesis cycle is inconsistent with such

evidence as there may be for a recent disturbance (~5 Myr) of the Oort cloud, namely the apparent non-equilibrium distributions of perihelia¹ and $1/a$ (ref. 9) and the enhancement of the short-period comet population^{10,11} (compare ref. 12). It also discounts the generally high glacial, magnetic and orogenic activity on Earth within this period and the Sun's recent passage through Gould's belt^{10,13}. However, the most serious problem is reconciling the approximately constant time-averaged cratering rate for the last ~3,000 Myr (ref. 14) and the stability of the proposed system.

The long-period comet system is dynamically unstable, over the lifetime of the Solar System, against the perturbing action of molecular clouds¹⁵⁻¹⁷. Since the proposed companion star has an aphelion distance $Q \approx 180,000$ AU (that is, $P \approx 30$ Myr) as against ~40,000-50,000 AU for long-period comets, one expects its orbit to be unstable *a fortiori*. On the impulse approximation the specific energy change ΔE of the companion resulting from a change Δv in its velocity v relative to the Sun is

$$\Delta E = \frac{1}{2}(v + \Delta v)^2 - \frac{1}{2}v^2$$

or

$$\Delta E = v \cdot \Delta v + \frac{1}{2}(\Delta v)^2 \quad (1)$$

Considering only the random component $v \cdot \Delta v$, Oort¹⁸ derived an expression for the specific energy fed into a body of separation Q from the Sun in $t \times 10^9$ yr, as a result of encounters with

Table 1 Energy injected by various perturbers

Perturber	Energy, Δv^2 [(cm s^{-1}) ²]
GMCs with $M = 2 \times 10^5 M_\odot$, $\nu = 40 \text{ kpc}^{-3}$	3.8×10^9
Molecular clouds with $M = 2 \times 10^4 M_\odot$, $\nu = 400 \text{ kpc}^{-3}$	9.6×10^9
Stars	4.2×10^9
Σ	17.6×10^9
Energy required for escape	0.9×10^8

point masses. Modifying¹⁵ to allow for the effect of penetrating encounters with molecular clouds of mass M solar masses, radii R AU and number density $\nu \text{ kpc}^{-3}$, one finds

$$\Sigma \Delta v^2 = 740 \nu \left(\frac{M}{2.21 \times 10^5 M_\odot} \right)^2 \times \left(\frac{Q}{R} \right)^2 \left(\frac{t}{4.5 \times 10^9 \text{ yr}} \right) 10^8 \quad (\text{cm s}^{-1})^2 \quad (2)$$

The energy injected by various perturbers over $4.5 \times 10^9 \text{ yr}$ is listed in Table 1. According to Sanders¹⁹ recent determinations of the mass of the molecular cloud system agree to within a factor ~ 2 , whilst the column density of molecular clouds is $\sim 5 M_\odot \text{ pc}^{-2}$ at the solar distance, the mass-averaged mean mass of giant molecular clouds (GMCs) being $\sim 5 \times 10^5 M_\odot$. The first two entries in Table 1 each correspond to a column density $\approx 1 M_\odot \text{ pc}^{-2}$ and are therefore below the extreme lower limit of mass allowed by the data. There is also considerable substructure within GMCs, which adds substantially to the energy injected: thus GMCs ($\sim 10^5$ – $10^6 M_\odot$) appear to comprise aggregates of molecular clouds ($\sim 10^4 M_\odot$). We consider here three extreme models which are likely to encompass all reasonable possibilities: GMCs are uniform throughout their interiors (rows 1+3); GMCs do not exist, only molecular clouds (rows 2+3); GMCs comprise molecular clouds as substructure (rows 1+2+3). It seems that even with the most conservative assumption the energy injected into the Sun/Nemesis binary by molecular clouds and stars is at least an order of magnitude greater than its binding energy and that the system could not survive in its postulated state for $4.5 \times 10^9 \text{ yr}$. Most probably, the sum of the injected energies is ~ 200 times that required to eject the companion star from the Solar System. The probable survival time can be found from equation (1) by equating the energy input to the binding energy whence it is found that, in the most likely case of rows 1+2+3, the formal survival time is $\sim 50 \text{ Myr}$. Considering only the effect of uniform GMCs (rows 1+3), the survival time is found to be $\sim 100 \text{ Myr}$. The rate of energy input is decreased by a factor ~ 1.5 – 2 allowing for the exponential z -distribution of molecular clouds¹⁶, and increased by factors ~ 2 and ≥ 2 allowing for gravitational focussing and a more gaseous past Galaxy respectively¹⁷.

The dissolution time τ of a binary system has been derived also by Chandrasekhar²⁰ neglecting the random component in (1) and considering only the systematic unbinding term $\frac{1}{2}(\Delta v)^2$. Adjusting his formula slightly to allow for the high eccentricity of Nemesis, one finds

$$\tau \approx \frac{7 \times 10^{13}}{\nu M Q^{3/2}} \times 10^9 \text{ yr} \quad (3)$$

yielding similar survival times, for example $\tau \sim 100 \text{ Myr}$ for uniform GMCs with $\nu = 40 \text{ kpc}^{-3}$ and $M = 2 \times 10^5 M_\odot$. Putting these two effects together (equation (1)), it is evidently unlikely that the Sun–Nemesis system will survive the Galactic environment for more than two or three revolutions.

In addition to the disruptive influence of encounters with molecular clouds and stars, the somewhat lesser effect of the Galaxy's smoothed-out mass distribution has to be considered. The disturbing potential due to the Galaxy is $\phi = -\frac{1}{2}(\alpha x^2 + \gamma z^2)$, with $\alpha \sim 1.6 \times 10^{-30} \text{ s}^{-2}$, $\gamma \sim -4.8 \times 10^{-30} \text{ s}^{-2}$, in a heliocentric

rotating coordinate system with x -axis directed towards the galactic anticentre, y -axis in the direction of rotation and z -axis towards the north galactic pole²¹. The critical Hill surface, beyond which orbits around the Sun are unstable, is roughly a tri-axial ellipsoid with $x_{\text{max}} \approx 300,000 \text{ AU}$, $y_{\text{max}} \approx 200,000 \text{ AU}$, $z_{\text{max}} \approx 150,000 \text{ AU}$. Thus with aphelion $\sim 180,000 \text{ AU}$, the orbit of Nemesis is barely if at all stable. Take the orbit to be roughly rectilinear, let χ represent the angle between orbit and x -axis, and consider the perturbing forces acting on the companion while its radius vector $r > a \approx 10^5 \text{ AU}$, which will hold for about half the orbital period. Over this time, the x -component of the galactic field satisfies $\ddot{x} = \partial\phi/\partial x \approx \alpha a \cos \chi$, whence $|\dot{x}| \geq (\alpha a \cos \chi) T/2$. With $a \approx 1.5 \times 10^{18} \text{ cm}$, $T \approx 9 \times 10^{14} \text{ s}$, $\cos \chi \approx \frac{1}{2}$ one obtains $|\dot{x}| \geq 0.05 \text{ km s}^{-1}$. Likewise $|\dot{z}| \geq 0.15 \text{ km s}^{-1}$. These perturbations should be compared with the aphelion velocity of Nemesis, $\sim 0.03 \text{ km s}^{-1}$, and the circular velocity at $180,000 \text{ AU}$, $\sim 0.07 \text{ km s}^{-1}$. It is clear that the velocity vector of the companion star will be grossly perturbed around its aphelion: the binary system would not in general maintain the high eccentricity necessary for Oort cloud perturbations and indeed might not survive even a single revolution. Thus, the Galaxy's smoothed-out mass distribution is also an important factor, though if one were to (1) overlook molecular cloud perturbations; and (2) adopt a binary configuration with major axis close to the galactic plane, it is possible such a system might survive for $\geq 1 \times 10^9 \text{ yr}$. Note also that the proposed binary characteristics are very rare or absent amongst observed systems. Thus among binaries with solar type primaries, only $\sim 1\%$ have periods in excess of 0.3 Myr , the computed periods amongst common proper motion pairs ranging from 3.5 to $820,000 \text{ yr}$ with mean $67,000 \text{ yr}$ and median $3,100 \text{ yr}$. Furthermore, only $\sim 3\%$ of binaries have eccentricities ≥ 0.75 . These facts are of course consistent with disruption by molecular clouds and the Galaxy.

Received 3 July; accepted 22 August 1984.

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Dynamical constraints on the mass and perihelion distance of Nemesis and the stability of its orbit

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It has been suggested^{1,2} that the observed periodic extinction of species at intervals of 26 Myr (ref. 3) may be catalysed by a hypothetical stellar companion of the Sun, Nemesis, with an orbital period of 26 Myr . The passage of a stellar companion through the inner comet cloud⁴ will fill the loss cone of these comets and cause a comet shower to enter the planetary system. It has been estimated that about 20–30 comets will hit the Earth during a shower, which

lasts less than a million yr. Such Earth impacts would produce considerable environmental stress and may lead to widespread extinction of species⁴. I now investigate the effect of Nemesis on the comets in the inner comet cloud. This is used to determine the minimum required mass of Nemesis and its minimum required perihelion distance. The effect of passing stars on the stability of the orbit of Nemesis is investigated, and the probability of its having passed within the planetary system estimated.

My previous calculations⁴ were for a passing star in a hyperbolic orbit with an impact velocity of 30 km s^{-1} , which is very much greater than the orbital velocity of a long-period comet. This allowed an impulse approximation to be used. However, Nemesis has an orbit similar to that of the comets in the outer, steady-state or Oort comet cloud, so its orbital velocity is similar to that of the comets which it perturbs. A series of exact three-body calculations are used to determine the amount by which Nemesis perturbs the orbits of the comets in the inner cloud.

I consider comets having semimajor axes of 4,000 AU, which is about the maximum semimajor axis of the comets needed to produce a death shower. In the computer calculations, Nemesis is assumed to be a black dwarf with a mass of either $M_N = 0.05 M_\odot$, or $M_N = 0.005 M_\odot$. If the orbital period of Nemesis is 26 Myr and its mass is $0.05 M_\odot$, the semimajor axis of its orbit is $a_N = 89,200 \text{ AU}$ by Kepler's Third Law.

Because of the large semimajor axis of its orbit, Nemesis crosses the orbits of comets having semimajor axes of 4,000 AU at very nearly the parabolic speed. This means that the pericentre passage of Nemesis can be well approximated by modelling it as an object in a hyperbolic orbit with a velocity at infinity of 0.1 km s^{-1} , which is an order of magnitude less than its orbital velocity at pericentre passage. This approximation allowed me to use my massive, very accurate computer code which was originally applied to encounters between a binary system and a stellar intruder (refs 5, 6 and refs therein). The calculations used a representative comet orbit with a semimajor axis $a_c = 4,000 \text{ AU}$ and an eccentricity $e_0 = 0.999$ (see Table 1).

The loss cone comets are long-period comets which pass within the orbits of Jupiter and Saturn at perihelion. Such comets are lost by their ejection into hyperbolic orbits in a time comparable with the original orbital period of these long-period comets^{5,6}. They are not present unless perturbations by passing stars have deflected fresh comets into the loss cone within the previous orbital period. The latter situation occurs in the steady-state for comets in the classical Oort cloud⁴ (comets with semimajor axis, $a_c > 2 \times 10^4 \text{ AU}$). Comets with semimajor axis $< 2 \times 10^4 \text{ AU}$ only have their loss cones filled episodically by passing stars. An intense comet shower enters the planetary system whenever a close stellar passage occurs⁴.

To fill the loss cone of the comets in the inner cloud requires that the mean change in their pericentre distance $\langle \Delta q \rangle$ exceed the semimajor axis of the orbit of Saturn or $\langle \Delta q \rangle > 9.5 \text{ AU}$. Table 1 shows that for a Nemesis mass of $M_N = 0.05 M_\odot$ and for comets with semimajor axes $a_0 = 4,000 \text{ AU}$, this requires that the closest approach of Nemesis, its pericentre distance, be $< 2.6 a_0 = 1.04 \times 10^4 \text{ AU}$. Since the semimajor axis of Nemesis is $a_N = 89,200 \text{ AU}$, this requires that its orbital eccentricity be at least $e_N \geq (1 - R_{\min}/a_N) = 0.88$.

The orbit of Nemesis has been greatly perturbed by passing stars, so the probability of its having a given eccentricity is dictated by conditions of statistical equilibrium rather than by its initial eccentricity. The probability of its having an eccentricity e or greater at some arbitrary time is given by⁴

$$P_e = (1 - e^2) \quad (1)$$

For $e = 0.88$, we note that $P_e = 0.23$. Invoking the Ergodic Hypothesis that a time average for one object produces the same distribution as an ensemble average over many objects, we anticipate that $\sim 23\%$ of the time its orbital eccentricity is high enough for Nemesis to induce an intense comet shower at its pericentre passage. For a fixed value of Nemesis's pericentre distance q_N , the mean change in the pericentre distance of the comets with $a_c = 4,000 \text{ AU}$ scales as

Table 1 Relaxation of comet orbits by Nemesis

p/a_0	$R_{\min}/a_0$	$\Delta q/(\text{AU})$	N
$M_N = 0.05 M_\odot$			
2.5	0.134	84.8	100
5.0	0.531	134	551
9.0	1.68	149	551
10.0	2.06	79	551
11	2.47	12.8	551
12	2.91	4.1	100
15	4.41	1.7	100
20	7.41	0.7	100
$M_N = 0.005 M_\odot$			
5.0	0.554	2.3	551
9.0	1.75	7.4	551
10.0	2.14	1.3	551
11.0	2.57	0.54	551

$V = 0.1 \text{ km s}^{-1}$; $a_0 = 4 \times 10^3 \text{ AU}$; $e_0 = 0.999$. Impact parameter p , and closest approach $R_{\min} = q_N$ are shown in units of the semimajor axis of the comet orbit. $\langle \Delta q \rangle$ is the mean change in the pericentre distance of the comets.

Table 2 Maximum perihelion distance of Nemesis

$M_N/M_\odot$	$q_{\max}(a_0)$	$q_{\max}(\text{AU})$	$e_{\min}$	P_e	$1 - F_N$
0.015	1.7	6,800	0.92	0.15	0.12
0.02	2.1	8,400	0.91	0.17	0.13
0.05	2.6	10,400	0.88	0.21	0.16
0.10	4.0	16,000	0.85	0.27	0.20
0.20	7.4	30,000	0.66	0.60	0.40

$e_{\min}$ is the minimum orbital eccentricity of Nemesis required by $q_{\max}$. P_e gives the fraction of the time during which its eccentricity exceeds $e_{\min}$.

$$|\Delta q_c| \propto \frac{M_N^2}{M_\odot + M_N} \quad (2)$$

in the impulse approximation. In this approximation, we expect that a Nemesis with $M_N = 0.005 M_\odot$ would produce a $|\Delta q_c|$ about 96 times smaller than a Nemesis with $M_N = 0.05 M_\odot$. The scatter in the exact numerical results of Table 1 are fairly large, but they indicate that the lower-mass Nemesis produces a $|\Delta q_N|$ about twice that predicted by scaling from $M_N = 0.05 M_\odot$ using the impulse approximation.

Applying the scaling law given by equation (2) to the data in Table 1, we can find $|\Delta q_c|$ for other possible masses of Nemesis. Table 2 shows, as a function of M_N , the maximum value of q_N that would still allow the perturbations by Nemesis to fill the loss cones at pericentre passage (that is, still result in $|\Delta q| \geq 9.5 \text{ AU}$).

If we correct for the small breakdown in the scaling law indicated by the computer calculations for $M_N = 0.005 M_\odot$, we see that a Nemesis with a mass $M_N = 0.01 M_\odot = 10$ Jupiter masses may be close to the minimum required mass, but a Nemesis with $M_N = 0.005 M_\odot$ is well below the minimum mass.

When the loss cone is filled, the fraction of the comets of semimajor axis a_c which have pericentre distances of q_c or less is $F_1 = 2q_c/a_c$, for $q_c \ll a_c$ (ref. 4). Here F_1 is also the fraction of the comets of semimajor axis a_c which are lost per orbital period due to the perturbations by Jupiter and Saturn if $q_c \leq 9.5 \text{ AU}$. The fraction of the comets of a given semimajor axis surviving N loss cone fillings is given by

$$F_N = [1 - (2q/a_c)]^N \quad (3)$$

If the loss cone of the inner cloud comets were filled at every perihelion passage of Nemesis for the past $4.6 \times 10^9 \text{ yr}$ then $N = N_0 = 177$ assuming the orbital period of Nemesis has remained constant at $2.6 \times 10^7 \text{ yr}$. For comets with $a_c = 4,000 \text{ AU}$ and $q_c \leq 9.5 \text{ AU}$, $F_N = 0.43$ for $N = 177$. In this case, more than half these comets would be lost. However, the orbit of Nemesis is severely perturbed by passing stars. The factor P_e in Table 2 is the fraction of the time that its orbit is eccentric enough and

Table 3 Change in orbit of Nemesis due to stellar intruders

p/q_N	N_i /Encounter	$\Delta q_N/q_N$	$\Delta q_N/\text{AU}$	$\Delta p/p_N$
0.1	0.46	0.717	3,200	0.1333
0.2	1.8	0.205	914	0.0559
0.5	11.5	0.080	357	0.0413
1.0	46	0.049	219	0.0102
1.5	104	0.044	198	0.0103
2.0	184	0.020	89	0.0025

p/a_N is the impact parameter in units of the semimajor axis of the orbit of Nemesis. N_i shows the average number of stellar intruders that pass within distance p/a_N of the Sun within one orbital period of 26 Myr. This was scaled from the results given in ref. 4. Δq_N is the change in the pericentre distance per encounter listed in units of the original pericentre distance and in units of AU. $\Delta p/p_N$ gives the average fractional change in the orbital period of Nemesis due to the passage of a single solar mass intruder.

its perihelion distance short enough to allow Nemesis to fill the loss cone of comets with $a_c = 4,000$ AU. The number of loss cone fillings is $N = N_0 P_c = 177 P_c$ for these comets. The last column of Table 2 shows $F_L = 1 - F_N$, the fraction of the comets in the inner cloud that have been lost.

I simulated 5,400 encounters between solar-mass stars and the Sun–Nemesis system at an impact velocity of 30 km s^{-1} . Here Nemesis is assumed to have a mass of $0.05 M_\odot$, its orbital semimajor axis is taken as $a_N = 89,200$ AU, and its eccentricity is taken as $e_N = 0.95$. Because the impact velocity is much higher than the orbital speed of the Nemesis–Sun system, gravitational focusing is not important and the closest approach of a stellar intruder to the Sun is very nearly its impact parameter. At each of six different impact parameters, p , 900 different simulations were made. None of these 5,400 encounters led to the intruder knocking Nemesis into a hyperbolic orbit. (However, molecular clouds may perturb Nemesis more than passing stars^{7,8}.)

While most stars are less massive than the Sun and would produce smaller perturbations per encounter than shown in Table 3 (the perturbations in period are proportional to the intruder mass), the large number of stellar encounters per orbital period of Nemesis ensures that at least one star having a mass equal to or larger than the Sun passes within distances (p/a_N) = 0.5 per orbital period. This requires that the average minimum change, $\langle \Delta q_N \rangle$, in pericentre distance q_N suffered by Nemesis per orbital period is at least as large as that resulting from an individual star of solar mass passing within distance $p/a_N = 0.5$. Dividing the value of Δq_N in Table 3 by the value of q_{max} in Table 2, we see that the variation in $\Delta q_N/q_N$ per orbital period of Nemesis must be at least 5% for $M_N = 0.015 M_\odot$ and about 2% for $M_N = 0.1 M_\odot$. If Nemesis has a low mass, we can expect fairly large variations in the strength of the comet showers at consecutive perihelion passages. Because of the large number of intruders, we can expect at least one or more intruder of solar mass to approach within distance $p/a_N = 0.5$ of the Sun during each orbital period of Nemesis. The fractional change in the period of Nemesis per orbital period is at least 4%. This can either be an increase or a decrease in the orbital period. After $N = 10$ revolutions the walk-away change in period should be at least 4% $N^{1/2} = 13\%$.

A more disastrous situation than a comet shower would arise if Nemesis were perturbed into an orbit that takes it into the planetary system. In this case, it may strip some planets directly from the Solar System. It would leave the remaining planets in highly eccentric orbits which would produce a fast-moving, dangerous situation⁹.

By the Ergodic Hypothesis the time-averaged properties of one object follows the same distribution as an ensemble average over many objects. The fraction of the time that Nemesis spends with a pericentre distance of q_N or less is $F_1 = 2q_N/a_N$. For $q_N = 40$ AU and $a_N = 89,200$ AU, $F_1 = 9 \times 10^{-4}$. As the average change in the pericentre distance, $\langle \Delta q_N \rangle$, of Nemesis per orbital period is much larger than the value of q_N considered, the probability of its entering the planetary system per pericentre

passage is 9×10^{-4} . For $N = 177$ pericentre passages, the total probability that it has entered the planetary system is

$$P_N = 1 - (1 - F_1)^N = 1 - (1 - 2q_N/a_N)^N = 0.15 \quad (4)$$

Obviously, this catastrophe did not occur, but the probability is high enough to be interesting.

Nemesis itself may be responsible for the termination of the planetary system at Pluto. If comets formed in the solar nebula just outside the orbit of Pluto, they could have been ejected into the comet cloud by Nemesis. However, the comets may have formed in the outer part of the proto-Sun in the inner comet^{4,10–12}.

Nemesis may not be the only massive object in the Oort Cloud. However, the fact that the planets have survived in nearly planar, circular orbits indicates that they have not been greatly perturbed since the dissipation of the solar nebula. It is unlikely that any massive object has plunged through the planetary system since its formation. A massive object with a semimajor axis of 2×10^4 AU, that is, one at the inner edge of the Oort or steady-state comet cloud, would have a loss cone $F_1 = 2q/a_0 = 4.5$ times larger than that of Nemesis. Its orbital period would be about 0.1 that of Nemesis, so it has made $N \approx 1.7 \times 10^3$ orbital revolutions. Using equation (4) we find that the probability of its having entered within the orbit of Saturn to be about 80%. The lack of apparent damage to the planetary system points to the unlikelihood of their being any massive objects in the inner edge of the Oort Cloud. A Nemesis with a semimajor axis of 9×10^4 AU is dangerous to life on Earth, but a Nemesis at the inner edge of the Oort Cloud would probably have produced a catastrophe of truly cosmogonical proportions.

Received 24 April; accepted 4 July 1984.

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How stable is an astronomical clock that can trigger mass extinctions on Earth?

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The periodicity in mass extinctions observed in the fossil record^{1,2} may be driven by an astronomical clock consisting of a companion star to the Sun^{3,4}. Each perihelion passage of the companion star would result in an enhanced rate of arrival of comets in the inner planetary system, some of which could collide with the Earth and perturb the atmosphere strongly enough to cause a catastrophic extinction of many, if not most, species^{5,6}. A similar periodicity has been observed in the cratering rate on the Earth^{7,8}. On Earth, the dating of both mass extinctions and crater formation is subject to observational uncertainties. In the heavens, too, we should not expect the 'double star clock' to be perfect, because of the influence of the galactic tidal force field and the perturbations of passing stars. On the basis of orbit calculations reported below, I expect the irregularity in the period of revolution over the past 250 Myr to be at least 10%, and more likely around 20%. This suggests that even completely accurate dating of mass extinctions and crater impacts should not be expected to yield perfectly sharp peaks in the power spectrum of a Fourier analysis.

The present results have been derived from an extensive series of numerical orbit calculations (including 10^7 stellar encounters) the details of which will be published elsewhere. These calculations take into account the perturbations of passing field stars as well as the galactic tidal field on the orbit of a double star; additional perturbations are caused by occasional passages close to molecular clouds and through spiral arms. The first two types of perturbations, stars and galactic tides, act continuously. The galactic tidal field has a nearly constant strength over the orbit of the Sun, and passing stars appear in large numbers: every million years the Sun encounters many different stars some of which actually pass through the orbit of the solar companion. Because of their intermittent character, the effects of molecular clouds and spiral arms are less important on a 250 Myr time scale on which the extinction record is well documented; these will be discussed in more detail elsewhere.

The strongest component of the tidal force acts perpendicular to the galactic plane, where the gradient of the galactic force field is steepest, and has a restoring character. The tidal force in the direction to the galactic centre is smaller by about a factor of six, and tends to pull a wide double star further apart. This radial force is composed of two parts, one of which stems from the radial gradient in the galactic force field and the other from the centrifugal force in the coordinate system rotating with the Sun in its path around the Galaxy. Another important effect in the rotating frame of reference is the Coriolis force acting in the galactic plane perpendicular to the motion of the companion. I have modelled all these effects in a linearized approximation, which is sufficiently accurate⁹.

Each field star passing close to the double star exerts a perturbing force which acts over a time interval much shorter than the orbital period of the binary, by a factor typically of the order a few hundred. Therefore, I have used the impulsive approximation, where the perturbing force is taken to act instantaneously at one point in the binary orbit. This approximation saves a factor of nearly 100 in computer time, and gives 1 Myr worth of orbit integration, featuring about 100 stellar encounters, every few seconds (rather than minutes¹⁰) of central processing unit time on a VAX 11/780 computer.

To understand the relative importance of both types of perturbations, I consider first the galactic tidal field only, neglecting passing stars, and orient the major and minor axes of the orbit along two of the three principal axes of the tidal field. For each of these six choices, I have computed many orbits, all starting with the same eccentricity $e = 0.7$, but with different values for α , the length of the semi-major axis, to find an orbit with a periodicity of 26 to ~ 28 Myr (Kepler's third law is a poor approximation in the presence of strong tidal forces). The numerical values for the components of the tidal force have been computed from recent estimates at Oort's A and B constants ($A = 16$ and $B = -11$, in $\text{km s}^{-1} \text{ kpc}^{-1}$, refs 11, 12) together with the inferred mass density in the solar neighbourhood which includes the unobserved galactic disk material³, while the Coriolis forces follow from the inferred galactic rotation frequency^{11,12}.

Figure 1a shows the largest tidal perturbations which occur for orbits with their major axis perpendicular to the galactic plane and minor axis perpendicular to the direction of the galactic centre (so that the galactic tidal force is purely restoring, which shortens the orbital period and therefore requires a larger, less stable orbit to satisfy a given orbital period). Figure 1b shows the most regular orbit, which lies in the galactic plane with its major axis pointing to the galactic centre.

Orbits parallel to the galactic plane are not affected by the strongest component of the tides, and thus are more regular and have a smaller amplitude than orbits which feel the compressive but overstabilizing tidal field component perpendicular to the galactic disk. Both effects suggest that a search for the companion star might have a higher chance of success at lower galactic latitudes (even though crowding is worse there), because (1) the fossil record as well as crater ages are more compatible with the regularity of Fig. 1b than with Fig. 1a, and (2) the

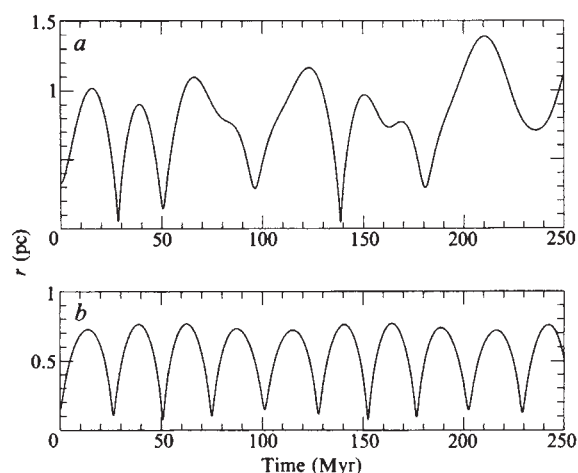


Fig. 1 The separation between the Sun and companion star under the perturbing influence of the galactic tidal field, in parsecs (1 pc $\approx$ 206,000 AU). *a*, For an orbit perpendicular to the galactic plane; *b*, for an orbit in the plane.

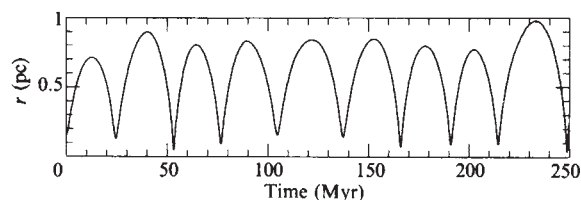


Fig. 2 The separation between Sun and companion star under the combined perturbing influences of passing stars as well as the galactic tidal field, for an orbit with the same initial conditions as in Fig. 1b.

smaller excursions in Fig. 1b make the orbit less vulnerable to perturbations by passing stars, which I now discuss.

I have immersed a double star in a field of passing stars, most of which cause only a tiny perturbation even when they cross the binary's orbit, because the total momentum transfer is proportional to the time spent in the encounter (typically only 0.1–1% of the orbital period). For the distribution of field stars I have accurately modelled the solar environment, using 10 discrete mass classes in the range 0.25 – $20 M_{\odot}$, each with their particular observed number density and velocity dispersion^{11,12}, with a total mass density of $0.06 M_{\odot} \text{ pc}^{-3}$ ($0.02 M_{\odot} \text{ pc}^{-3}$ of which resides in light stars of $0.25 M_{\odot}$, to model the unseen disk material; a choice of $0.05 M_{\odot} \text{ pc}^{-3}$ in the form of $0.1 M_{\odot}$ stars would have given a similar cumulative effect). The procedure for generating the properly weighted random distributions of orbital elements has been described previously¹⁰ using a Monte Carlo approach to obtain scattering cross-sections in the gravitational three-body problem.

Figure 2 shows an orbit which starts out with exactly the same initial conditions as in Fig. 1b, but which is now subject to stellar perturbations as well as to the galactic tidal field. Note the fluctuations in the time intervals between successive close approaches (perihelion passages) of the companion, spanning a range 23 to ~ 34 Myr. This suggests that even completely accurate dating of mass extinctions and crater impacts should not be expected to yield perfectly sharp peaks in the power spectrum of a Fourier analysis. A more sensitive test of the hypothesis of an unseen solar companion follows rather from the prediction of a tight correlation between individual periods of enhanced cratering rates and periods of mass extinction (several authors have argued that an alternative explanation can be given using the Sun's periodic motion perpendicular to the

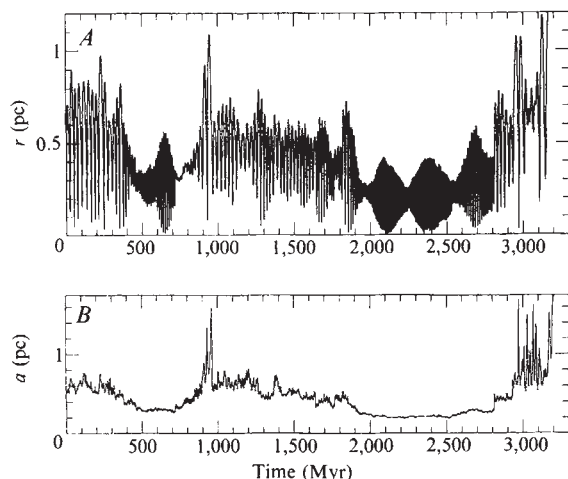


Fig. 3 *A*, The same orbit as in Fig. 2, followed until final dissolution around AD 3,200,000,000. *B*, The evolution of the semi-major axis of the orbit in *a*. The noise in the perturbations has a fine structure down to 0.01 Myr.

galactic plane^{8,14}; however, it is hard to understand why both extinctions and cratering seem to occur when the Sun is furthest away from the galactic plane^{1,7}. Although the intermediate time intervals cannot be predicted individually, their probability distribution can be obtained from orbit calculations, which thereby provide additional constraints.

Also note in Fig. 2 the significant scatter in the distance of closest approach. The number of comets directed into the inner planetary system at these times is strongly dependent on this distance, although for small perihelion distances a saturation effect might occur if the loss cone is filled completely^{3,4}. This could naturally explain why some mass extinctions have been so much more dramatic than others^{1,2}. The term 'loss cone' is used in analogy with plasma physics, where a mirror machine has a cone-shaped region in velocity space from which the plasma can escape. For the gravitational application the region in velocity space from which comets are lost is really bounded by a loss hyperboloid-of-one-sheet¹⁵; this is sufficiently less euphonious than the term loss cone is preferable as long as it is understood to refer to a strictly hyperboloid geometry.

I have computed several hundred orbits, each starting with a revolution period 26 to ~28 Myr, and with an initial distribution isotropic in space. As mentioned above, the orbits parallel to the galactic plane live longer than those perpendicular to the plane. About half of the total sample of orbits dissolved within 1,000 Myr, in agreement with analytical estimates¹⁶⁻¹⁸. A more quantitative discussion has to include the complicated dependence of the dissolution rate on the orientation of the orbit with respect to the Galaxy, and lies beyond the scope of the present letter. These half lives decrease if the effects of molecular clouds and spiral arms are taken into account, but the decrease is sensitively dependent on the distribution of molecular clouds, which is observationally less well determined than that of the field stars. A realistic guess for the final life time would be a value larger than 500 Myr, because (1) the Sun is, at present, ~1 kpc closer to the galactic centre than on average, and (2) the Sun has an unusually low velocity perpendicular to the galactic plane at present (Wielen, personal communication). Both effects strongly diminish the influence of giant molecular clouds which are concentrated towards the galactic plane and towards the galactic centre, in contrast with results by other authors¹⁸⁻²⁰. A detailed analysis of orbit calculations which include these effects will be published elsewhere.

With a half life of 1,000 Myr, survival times of a few 1,000 Myr still occur frequently. Figure 2 shows a double star orbit which lasts for 3,200 Myr, as plotted in Fig. 3A, to illustrate the richness of the spectrum of perturbations. The final breakup of the double star occurs after a random walk in orbital parameter space,

through the combined influence of more than 100,000 individual encounters with field stars. These perturbations can be seen more clearly in Fig. 3B, which plots the semi-major axis a of the orbit. Typically, every 10,000 yr another field star begins an encounter (included in the numerical calculations whenever it occurs within a distance of five times the semi-major axis) each of which lasts for a 100,000 yr. Occasionally a large jump occurs, which can be caused in any of three different ways; the passing star can be extra massive, come extra close or move extra slowly, in each way increasing the total momentum transfer.

The noise in Fig. 3B is strongly dependent on the size of the orbit. For $a < 0.3$, on the right-hand side of Fig. 3B, the jitter has died down by nearly an order of magnitude. This is because fewer stars pass close to a tighter orbit, and larger perturbations are required significantly to affect a tighter and therefore more energetic orbit. During these lulls in stellar perturbations, the galactic tidal field makes itself visible in the slow modulation of the eccentricity with a period of about 300 Myr, as can be seen in the rising and falling of the amplitude of the excursions in r on the right-hand side of Fig. 3A.

The closest encounter in Fig. 3A brings the companion to a distance of 800 AU from the Sun, by AD 2,100,000,000. Encounters much closer than that are rare during the lifetime of the Solar System, as I have found from orbit calculations starting at an initial separation of 0.1 pc (resulting in a half life of ~5,000 Myr). This suggests that a planetary system can indeed survive the presence of a distant solar companion, as the expected induced eccentricity for the orbits of the outer planets is < 0.01 (ref. 21), even if the companion would come in as close as 200 AU. A detailed study of the perturbations on the planets would nevertheless, be very interesting; it would probably not put very stringent restrictions on the hypothesis of a solar companion, but it might explain the ill-understood irregularities in the planetary system.

Figures 2 and 3 chart only one of the many possible future evolutions of the orbit of a solar companion. Each individual orbit calculation yields vastly different results, since the passing stars exert a stochastic perturbing force on the double star. These perturbations are unpredictable on a short time scale and difficult to treat analytically, even using the average for longer time scales, because of the complicated interference with the galactic tidal field. Each of the different future evolutions which I have calculated as starting with the initial conditions of Fig. 1b are equally likely to occur, as are many others starting from different initial conditions (but with the same initial orbital period of 26 to ~28 Myr).

The evolution of the hypothetical solar companion can be summarized as follows: the original orbital period at the time of the formation of the Solar System was probably in the range 1 to ~5 Myr, much shorter than the present value of 26 to ~28 Myr; and the final escape of our companion star might take place relatively soon, on a time scale of the order of 1,000 Myr.

After completing this work I received a preprint by Smoluchowski and Torbett²², whose calculations of tidal disturbances of comets agree with the present paper.

I acknowledge interesting discussions with many colleagues, especially Luis Alvarez, Walter Alvarez, John Bahcall, Haldan Cohn, Ivan King, Myron Lecar, Eugene Shoemaker, Fred Whipple, Roland Wielen, Richard Muller, Scott Tremaine and Alar Toomre. This work was supported in part by NSF grant PHY-8217352.

Received 24 April; accepted 15 June 1984.

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Orbital stability of the unseen solar companion linked to periodic extinction events

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Recent suggestions of astronomical causes for the closely periodic extinction events in the geological record¹ and the possible periodicities in the cratering record² have involved two basic ideas. First, it has been suggested^{3–5} that the oscillations of the Solar System through the galactic plane with semi-periods of the order of 30 Myr make possible close encounters with giant molecular clouds, or sub-units thereof, which are expected to scatter Oort cloud comets into the terrestrial zone. Second, it has been suggested^{6,7} that a low-mass, and as yet unseen, companion to the Sun, tentatively named Nemesis, is in an eccentric orbit ($e \sim 0.7$), such that perihelion passages bring it through the postulated dense inner Oort cloud⁸. Perturbations of cometary bodies there result in scattering of some 10^9 comets into the terrestrial zone. Those that impact the Earth are assumed to loft dust into the atmosphere, causing climatic chaos^{5,9}. To achieve the period that matches the best estimates for the intervals between extinction events, semi-major axes in the range 9×10^4 – 1×10^5 AU are required. Whether such orbits are indeed stable over the lifetime of the Solar System is not known. We present here evidence from three-dimensional numerical modelling that only orbits with a limited range in inclination with respect to the galactic plane are formally stable for the required length of time. Perturbations bypassing stars and molecular clouds may make even these orbits unstable.

Calculations were done using Cowell's method employing a fourth-order Runge–Kutta integration scheme in an inertial reference frame in orbit about the Galaxy, which is approximated as a point mass of $1.3 \times 10^{11} M_\odot$ at a distance of 8.22 kpc from the Sun to give a galactic rotation velocity of $\sim 250 \text{ km s}^{-1}$. Tidal perturbations in the radial direction due to the Galaxy as well as Coriolis forces are thus automatically included. Superimposed on these forces is the vertical component of the gravitational field of the galactic disk, taken from the results of Bahcall¹⁰ and given per unit mass as

$$f(z) = 2.565 \times 10^{-11} z - 1.66 \times 10^{-16} z^3 \text{ cm s}^{-2} \quad (1)$$

where z is in parsecs. Hence, the companion is subject to the 'gravitational compression shock' during its excursions through the galactic disk. Moreover, since the semi-period of this motion (~ 30 Myr) is comparable with the orbital period (26–29 Myr), resonant accumulation of the perturbations can become significant, eventually leading to disruption of the system.

The calculational procedure was as follows. The Sun–companion barycentre was given initial conditions correspond-

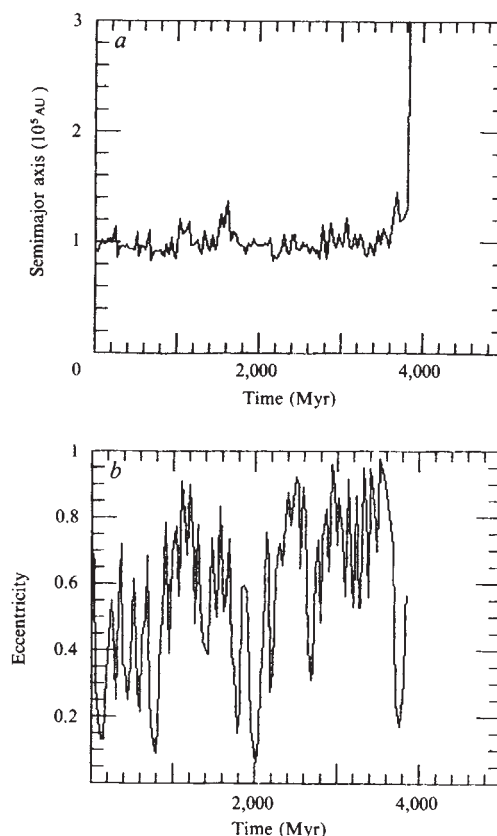


Fig. 1 *a*, Semimajor axis and *b*, eccentricity as functions of time for Nemesis in an orbit about the Sun inclined at an angle $i_0 = 40^\circ$ with respect to the galactic plane. System becomes unstable within the age of the Solar System.

ing to a circular orbit about the galaxy with an initial height above the plane equal to that expected for its oscillation amplitude (~ 100 pc). The initial inclination with respect to the galactic plane of the companion's orbit was adjusted to yield a resultant mean inclination, i , which was stepped from 0° to 180° in intervals of 10° . During the course of a typical run, the scatter in the inclination amounted to $\sim 10^\circ$. The companion and the Sun were then given initial conditions so as to produce a semi-major axis, a , such that the mean interval between perihelion passage was in the range 25–30 Myr and eccentricity, e , in the range such that perihelion passage occurred within the inner Oort cloud. To achieve the proper period, it was necessary to vary considerably the semimajor axis due to the strength of the perturbations relative to the solar attraction at the separation required. Values of 10^{-1} and $10^{-2} M_\odot$ for the mass of Nemesis were both investigated, with negligible differences in the results.

The results indicate that for inclinations $i \geq 30^\circ$ the orbits are unstable with the system being disrupted within the age of the Solar System. This is true for both prograde and retrograde orbits. A representative case is illustrated in Fig. 1 where a and e are plotted as a function of time for $i \sim 40^\circ$. Orbits with larger values of inclination than shown in Fig. 1 become unstable even sooner. For example, at $i \sim 90^\circ$, the system is disrupted within 500 Myr. For inclinations $i \leq 30^\circ$ the orbits appear stable although irregular as shown in Fig. 2 where a and e are again plotted as a function of time. Orbits with high galactic inclination are less stable than low inclination orbits since the vertical tidal field of the disk is 3 or 4 times stronger than is the radial galactic tide at the Sun's position. Thus, inclined orbits sense stronger perturbations than do orbits lying in the plane. This leads to more chaotic trajectories for the inclined case as can be seen by comparison of Figs 1 and 2. Hence, since the orbits with periods corresponding to extinction intervals verge on the margin of stability¹¹, the near-resonant match of orbital period with

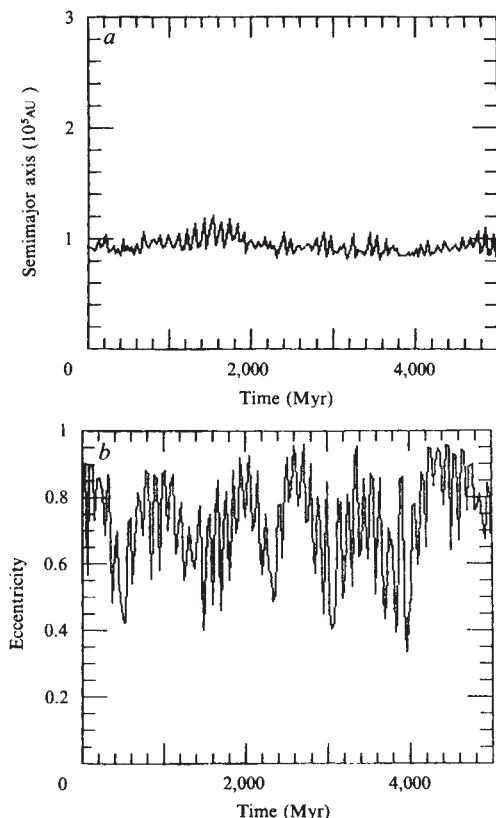


Fig. 2 *a*, Semimajor axis and *b*, eccentricity as functions of time for Nemesis with galactic inclination $i_0 = 30^\circ$. Orbit is formally stable for the age of the Solar System, although irregular.

the semi-period of the Sun's oscillation about the galactic plane leads to disruptive accumulation of perturbations.

Figure 3 shows a histogram of the inverses of the intervals between perihelion passage for the case shown in Fig. 2. It is clear that the intervals vary widely within limits greater than the 25–30 Myr inferred from the data. This chaotic nature of the permitted direct orbits makes the causal connection of periodic extinctions with regularly timed astronomical events less compelling. Retrograde orbits with $i < \sim 30^\circ$ seem, however, to be considerably more stable because in this case Coriolis forces tend to increase rather than decrease stability. This results in the range of the limits for intervals between perihelion passage being factors of 2–3 smaller than in the prograde case and within the limits of the data. Thus, the scatter in orbital period can be reduced by assuming a retrograde orbit for Nemesis. Hence, if Nemesis exists, it may be in retrograde orbit; but even then, strict periodicities should not be expected even in perfectly dated fossil records. These results indicate that orbits for the companion that are inclined at more than 30° to the galactic plane are not allowed and suggests that the search for Nemesis should be concentrated towards the plane of the galaxy. This limit on the range of permissible galactic inclinations excludes orbits in the plane of the ecliptic, which makes an angle of $\sim 60^\circ$ with the galaxy. It is not impossible, however, that an orbit originally in the ecliptic could have been perturbed to lie near the plane of the galaxy by close encounters of passing stars or molecular clouds.

Perturbations by passing stars or molecular clouds may make even the low-inclination orbits unstable. Stability analysis¹² of companion orbits subject to stellar perturbations and the galactic tides, while neglecting the 'gravitational compression shock' due to the vertical oscillations, indicates lifetimes for Nemesis of the order of 10^9 yr which is consistent with our analysis. In addition, the randomizing effect of stellar perturbations on the orbital elements will change low-inclination orbits into high-inclination ones leading to disruption. Perturbations by molecular clouds may, however, deliver the most serious blow

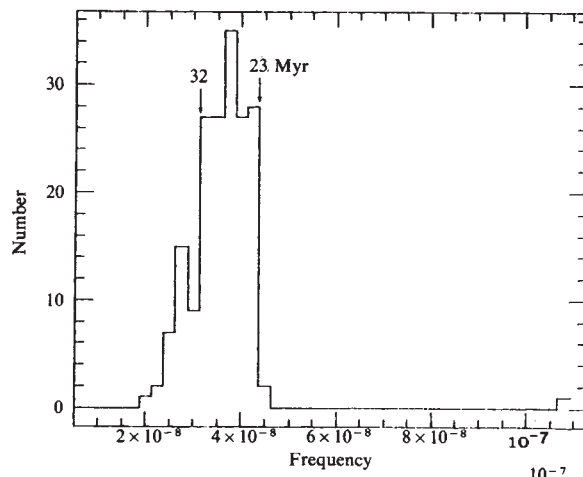


Fig. 3 Histogram of the inverses of the intervals between perihelion passage for prograde orbit of Nemesis for the case depicted in Fig. 2. Note the scatter in the intervals from 23 to 32 Myr. Thus strict periodicities should not be expected in fossil or crater records.

to stability. Numerical modelling¹³ of the effect of molecular cloud passages on cometary orbits indicates that for close encounters a large fraction of the orbits will be disrupted. Preliminary analysis from a current numerical study (Torbett, M. V. & Smoluchowski, R., in preparation) shows that, for the expected encounter distances from, and velocities relative to, $10^5 M_\odot$ clouds, a large fraction ($> 50\%$) of the orbits for Nemesis will be disrupted. This is in reasonable agreement with arguments based on balancing the tidal force of the cloud against the binding force.

Hence, at best, the existence of an appropriately positioned companion should be viewed as a highly temporary, low-inclination, and probably retrograde phenomenon. At worst, the instabilities induced by the galactic tides and molecular cloud passage make the companion hypothesis untenable.

M.V.T. acknowledged helpful discussions with Linda Stryker and Kirk Borne. R.S. is supported in part by NASA grant NSG 7505 suppl. 5.

Received 29 May; accepted 15 August 1984.

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Nitryl perchlorate as the essential intermediate in the thermal decomposition of ammonium perchlorate

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Because of its use as a solid propellant and more generally in pyrotechnic systems, the thermal decomposition of ammonium perchlorate (AP) has been extensively studied. The breakdown of this salt is complicated, an exceptional feature being that the low-temperature (~ 570 K) decomposition is incomplete

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(~30%), yielding a residual solid with a composition and crystal structure very similar to those of the original reactant. Extensive rate measurements and kinetic analyses¹⁻⁵ have produced two alternative general hypotheses of reaction mechanisms⁵, in which rate control has been attributed to steps involving the transfer of either a proton⁴ or an electron⁶. In the present study, we have found that the addition of various inorganic nitrates reduces the induction period to reaction and increases the extent of the low-temperature decomposition of AP. The measured rate of decomposition was close to that estimated for reaction involving nitryl perchlorate (NO_2ClO_4) as an intermediate and we have detected small quantities (~0.08%) of an oxidized nitrogenous species in partly reacted AP. We therefore propose a novel reaction mechanism in which NO_2ClO_4 is the essential intermediate in the thermal decomposition of ammonium perchlorate.

Our investigation of the possible role of oxidized nitrogen in promoting AP decomposition represents an attempt to characterize the chemical steps involved in breakdown of the relatively stable (at ~500 K) perchlorate ion, ClO_4^- . Khairtdinov and Boldyrev⁵ have pointed out that the present reaction, involving sublimation, occurs 300–350 K below the corresponding temperatures for reactions of other alkali perchlorates. Perchloric acid is sufficiently stable to survive sublimation and it is suggested that the low-temperature reaction may be incomplete due to accumulation of the relatively more stable acid hydrates, $\text{H}_3\text{O}^+\text{ClO}_4^-$, $\text{H}_5\text{O}_2^+\text{ClO}_4^-$, etc., on the walls of the channels eroded in the reactant crystals during decomposition. We have attempted to identify the intermediates involved in these reactions and the interactions that culminate in ClO_4^- disintegration.

It is known that ammonium salts of certain strongly oxidizing anions yield NH_4NO_3 on decomposition² and that the same compound, NH_4NO_3 , reduces the induction period to AP reaction. The formation of N_2 and N_2O during AP decomposition² can be explained by the intervention of nitrite and nitrate respectively, involving a mechanism generally similar to that recently proposed⁷ for the breakdown of $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$. At least one other type of additive containing oxidized nitrogen—nitrophenol-formaldehyde polymers—has been shown⁸ to accelerate AP breakdown. Oxidized nitrogenous species thus appear to merit particular consideration as intermediates.

We made isothermal kinetic studies of the decomposition of AP alone and with added NH_4NO_3 or $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, both of which readily melted on heating to reaction temperature. Comparative yield-time data for $\text{N}_2 + \text{O}_2$ evolution (in a conventional accumulatory apparatus³ using a 78 K refrigerant trap) during the low-temperature reaction² for consecutive experiments, in identical conditions, showed that both additives significantly reduced the induction period to reaction. Figure 1a shows typical results for the decomposition at 495 K of AP by itself and initially covered with molten NH_4NO_3 (most of which was rapidly volatilized out of the reaction vessel soon after the start of the reaction). The additive markedly reduced the induction period (from 120 to 40 min) and appreciably increased the reaction rate (by about 1.5 times) but it did not change significantly the final yield of product gases ($\text{N}_2 + \text{O}_2$).

Figure 1b shows the relative activities of four different inorganic nitrates in promoting the decomposition of AP powder at 503 K. Three of the additives (NH_4^+ , K^+ and Mg^{2+} nitrates) caused approximately similar reductions in the time to onset of reaction. The ammonium salt was the most effective of this group, however, in accelerating the overall rate of reaction, presumably because removal by distillation occurred less readily here and volatilization temporarily distributed the additive to all surfaces of the compacted powder reactant. Systematic variations in the proportion of added $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, between 1 and 25%, showed that the kinetic characteristics did not vary widely from the curve shown in Fig. 1b, but the total yield of gaseous products ($\text{N}_2 + \text{O}_2$) was always greater than that obtained from the pure salt. Silver nitrate was identified as being a particularly active catalyst for AP decomposition, causing the most rapid onset of reaction and the greatest increase in rate of decomposition.

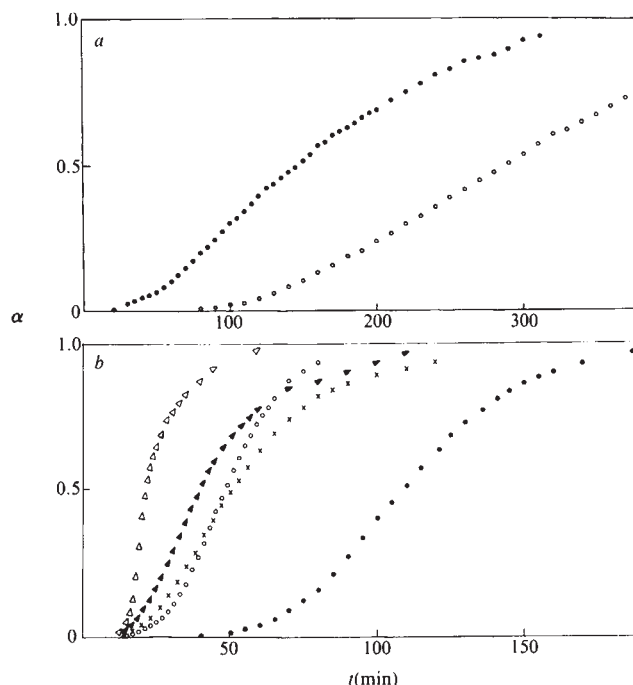


Fig. 1 *a*, Curves of comparative fractional reaction (α) against time for the isothermal (495 ± 1 K) decomposition of single crystals of AP measured for evolution of $\text{N}_2 + \text{O}_2$ (78 K trap) in identical conditions in a constant-volume system. One crystal was reacted 'as prepared' ($\circ$); the other was 'promoted' by the addition of NH_4NO_3 ($\bullet$), which resulted in a reduction of the induction period and an increase in the reaction rate. *b*, Comparative α -time curves for the decomposition of AP powder mixtures crushed with 10% (by weight) of various nitrates: NH_4NO_3 ($\circ$), $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ($\blacktriangle$), KNO_3 ($\times$), AgNO_3 ($\triangle$), and no additive ($\bullet$). All four additives reduced the induction period, accelerated the maximum rate of reaction and increased the extent of reaction. The silver salt was the most active catalyst. The reactant mixtures containing nitrate and AP were crushed in a pestle and mortar before reaction.

Microscopic inspection of large single crystals after partial ($\leq 10\%$) decomposition, initiated with either added NH_4NO_3 or $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, showed that the reaction was initially rapidly established over the entire surface of the AP reactant, in contrast to the well known development of individual, approximately hemispherical nuclei during reactions of unpromoted single crystals². The same conclusion was reached on scanning electron microscope examination of cleavage surfaces traversing reacted zones, exposed after the partial decomposition of large single crystals. Representative textures are shown in Fig. 2a,b for an exposed cleavage section of an AP crystal after 10% decomposition initiated by adding $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. A continuous layer of decomposed salt, with approximately constant thickness, has developed below the original crystal surface; this contrasts with the generation of separated reaction zones (nuclei) during reactions of the salt in the absence of additives: the edge of a discrete nucleus and a zone of un-nucleated surface are evident (Fig. 2c).

Previous observations³ have shown that the yield of gaseous products ($\text{N}_2 + \text{O}_2$) by a second successive AP decomposition is ~10% that of the first. Here we have repeated this observation and have also shown that the addition of NH_4NO_3 to a decomposed crystal, followed by the restoration of reaction conditions (510 K), results in a marked increase in the extent of the second reaction, to ~50%. This demonstrates the ability of the nitrate to promote AP decomposition.

Previous analytical measurements have detected NO_3^- in partly decomposed AP⁹ and in the sublimate². We have measured the amount of oxidized nitrogen in partially (50%) decomposed AP by two methods: first, by reaction with brucine¹⁰ in sulphuric acid, followed by spectrophotometric determination of absorption at 405 nm, and second, by the (less accurate)

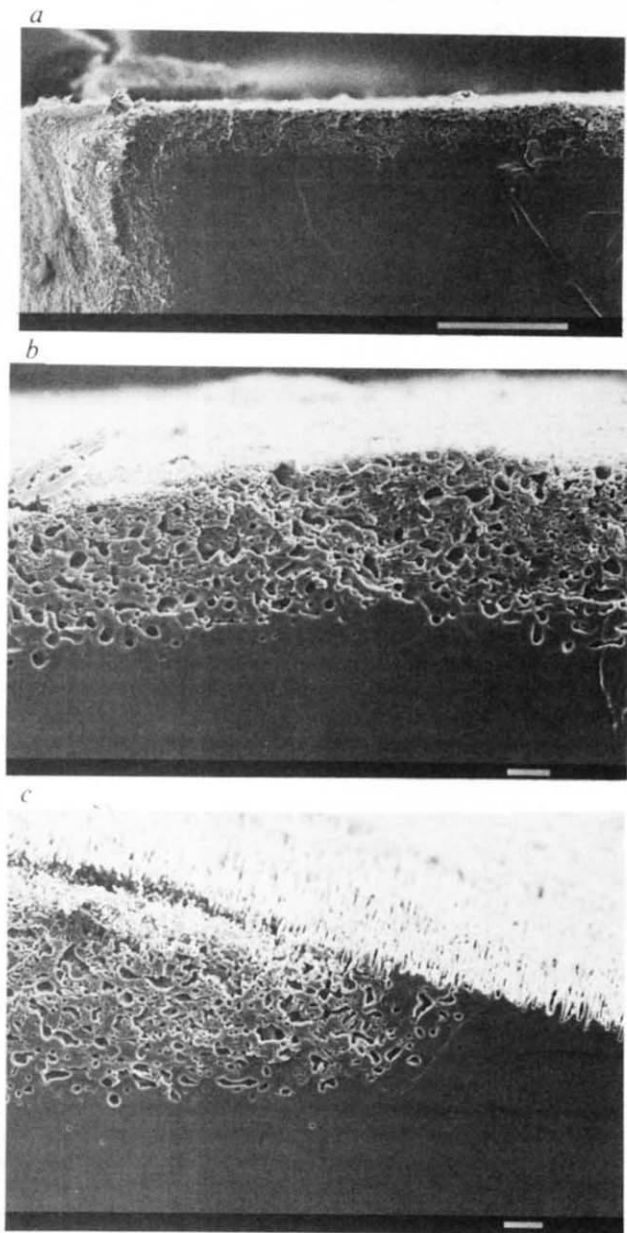


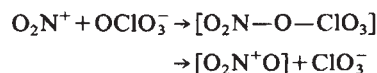
Fig. 2 Scanning electron micrographs of decomposition zones, revealed in section by cleavage of single crystals after 10% reaction. *a*, *b*, The reaction in this crystal was initiated by addition of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, which melted immediately on heating. The decomposed layer is of almost constant thickness and is developed below the total surface. *c*, In reaction of the pure salt, discrete nuclei were formed; the boundary edge of one of these is shown here. Although reaction has not occurred in the crystal beyond the nucleus, the surface is extensively grooved. Scale bars (white): *a*, 100 μm ; *b*, *c*, 10 μm .

colorimetric method as ammonia with Nessler's reagent (after removal of ammonia by boiling with alkali, followed by reduction with Devarda's alloy). While the low concentration of the active intermediate present precludes its specific identification (as NO_3^- or NO_2^+), repeated determinations gave reproducible evidence that the amount of oxidized nitrogen present corresponded to $0.08 \pm 0.04\%$ NO_2ClO_4 . (This choice of intermediate anticipates the conclusion reached in our mechanistic discussion below.) The quantities of oxidized nitrogen detected in the residual salt⁹ and in the sublimate² were less, corresponding to ~ 0.01 and $\sim 0.02\%$ NO_2ClO_4 , respectively.

It seems probable that nitrate present during decomposition under the oxidizing, acidic^{2,5} and probably dehydrating condi-

tions prevailing within the reaction zones must be in the form NO_2ClO_4 (ref. 11). Nitryl perchlorate, therefore, is identified as the reaction intermediate as it is unstable at reaction temperature^{11,12} and its presence can be maintained by dynamic equilibrium through ammonia oxidation by the active oxygen species resulting from ClO_4^- breakdown. NH_3 and/or NH_4^+ reacts with products of ClO_4^- disintegration to yield NO_2^+ which combines with a reactant anion and, on decomposition, the cycle continues.

In this mechanism, the difficult perchlorate ion breakdown step is explained by the formation and rupture of covalent bonds, as such perchlorates are less stable¹³ than the more ionic compounds:



The unstable $[\text{O}_2\text{N}^+\text{O}]$ may rapidly, perhaps concurrently, disintegrate, yielding $\text{N}^+\text{O} + \text{O}_2$ (or 2O); NOClO_4 is also a product of NO_2ClO_4 decomposition¹¹. The chlorate ion is known to be unstable under reaction conditions⁵ and capable of oxidizing NH_3 and/or NH_4^+ since NH_4NO_3 is a product of NH_4ClO_3 decomposition².

We estimate, by extrapolation of published¹² kinetic data, that decomposition of NO_2ClO_4 at 503 K would proceed from 20% to 80% in 0.10 min. At 50% reaction, our powder samples of AP reactant contain 0.08% NO_2ClO_4 . For this dynamic equilibrium concentration of intermediate and estimated reaction rate, we calculate, by direct proportions, that the time required to complete the low-temperature (503 K) rate process (30% AP weight loss) is ~ 70 min. The corresponding time estimated from the middle section of the curve for AP powder in Fig. 1*b* is ~ 85 min. In view of the uncertainties in our calculations, this agreement is unexpectedly close and provides strong evidence that the NO_2ClO_4 decomposition step is rate-controlling in AP decomposition. (The similarly estimated¹⁴ value for NH_4NO_3 decomposition is larger, and less attractive here because it does not result in ClO_4^- breakdown.)

In the kinetic argument presented above, we have not considered the phase within which the NO_2ClO_4 decomposes. If this intermediate is present as a solid, geometric controls may be expected to operate¹⁵. However, it is known that NO_2ClO_4 is volatile¹¹ at reaction temperature, though here confined within the pores of the reaction zone, and its melting point may be depressed by eutectic formation with the other perchlorates present (NH_4ClO_4 , HClO_4 , NOClO_4). It seems probable, therefore, that reaction occurs in local melted droplets (fusion nuclei¹⁵). Evidently, the concurrent oxidation of ammonia and the presence of hydrogen-containing products does not significantly affect the rate of decomposition of the NO_2ClO_4 intermediate. We also note that the activation energy for AP decomposition¹⁻⁴ is close to that^{11,12} reported for NO_2ClO_4 (130 kJ mol^{-1}), which provides a further indication that the same rate-controlling process operates in both reactions. This similarity is not, however, a proof of mechanism because the same value has also been reported¹⁶ for the breakdown of $\text{Cl}_2\text{O}_7(+\text{H}_2\text{O} \rightleftharpoons 2\text{HClO}_4)$, another covalent molecule. Thus, this temperature coefficient of reaction may be characteristic of the common step, the bond rupture: $\text{O}-\text{ClO}_3$.

The internal structures of zones of decomposed salt (nuclei), revealed by cleavage section after reaction, were identical in the pure salt and following initiation by NH_4NO_3 (Fig. 3*a* and *b*, respectively) or $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. This is evidence that the additive does not change the mechanism and is consistent with the view that promotion involves the conversion of NO_3^- to NO_2^+ , which thereafter participates in the chemical sequence described above.

Each pore in the reacted salt (~ 0.2 – $0.5 \mu\text{m}$ diameter) evidently resulted from the irregular progress of an active droplet of NO_2ClO_4 , and the rounding of these internal surfaces provides further indication of the participation of a fluid or molten reaction phase. The larger holes, up to $5 \mu\text{m}$ diameter, and

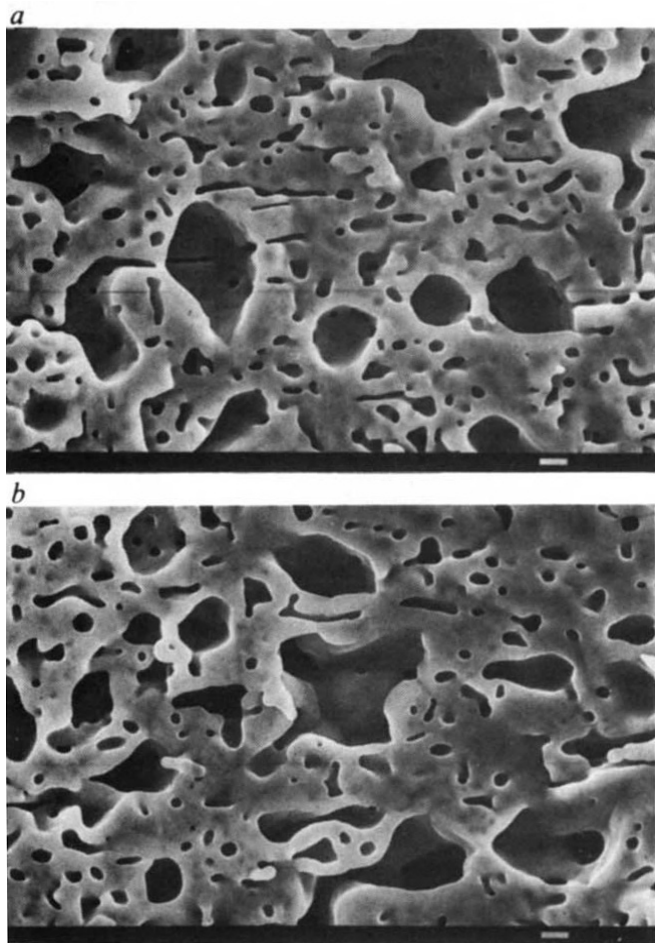


Fig. 3 Scanning electron micrographs showing cleavage sections of decomposed regions of AP in *a*, a pure crystal and *b*, a crystal in which the reaction was initiated by NH_4NO_3 . No difference in texture could be discerned in a series of careful comparisons of many areas of both types. We suggest that the texture of the decomposed salt is that of reactant remaining after penetration by the irregular migration of small particles of an active phase within or around which decomposition occurs, presumably including NO_2ClO_4 intermediate and possibly in the form of liquid. Enhanced reaction occurs when droplets coalesce, resulting in the larger pore spaces. Scale bars, $1.0\ \mu\text{m}$.

in the chemical change. Salt that is undecomposed after passage of active liquid droplets occurs because sites of enhanced reactivity have been removed and further intermediate cannot be produced in sufficient local concentration to generate a new reaction zone. The addition of NH_4NO_3 , however, was capable of initiating some further decomposition of the residue.

This mechanism can also explain the ability of certain compounds to act as catalysts for AP decomposition. NH_4ClO_3 yields² NH_4NO_3 , shown here to be active in promoting AP decomposition. Permanganate and chromium-containing⁷ anions may participate similarly by oxidizing ammonia, the reduced oxyanion being reoxidized through ClO_4^- breakdown.

We thank J. McCrae and R. Reed for advice on obtaining and interpreting the electron micrographs. M.A.M. thanks the Egyptian Government and the ORS Award Schemes for Scholarships held during this research.

Received 16 April; accepted 11 September 1984.

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South African modern beach sand and plate tectonics

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South America is the ideal continent for baseline studies on how the framework composition of modern sands relates to reconstruction of palaeoplates—it has well-defined active and passive margins, great contrasts in climate and relief and is free of continental glaciation (Fig. 1). I report here petrographical studies of its beach sands which show two active margin associations rich in volcanics and plagioclase—one along its Pacific coast and one along the Argentine coast—and two associations rich in quartz—a passive margin association from Argentina to Trinidad and a moderately, quartz-rich association along the Caribbean coast. Tectonic control is thus overwhelming but complications exist along the Argentine coast where a dry, narrow continent permits active margin sand to mantle a passive margin. Another surprising result is that quartz-rich sands border the crystalline rocks of much of the Serra do Mar in Brazil.

Sandstone has long been used¹ to help identify the tectonic setting of ancient basins for many reasons. It is present in almost every basin, including some of the very oldest, and it occurs in almost every sedimentary environment with the possible exception of the abyssal plains of some deep sea basins. Each grain also carries its own provenance history, which is easy to study with both microscope and microprobe. However, the idea that the petrographic study of 10–30 sandstone samples from an ancient basin, perhaps a sample of only 10^{-12} to 10^{-16} of its entire volume, can reveal a plate tectonic setting, and thus infer major crustal processes, may be considered audacious.

evidently linked by two or more pores, may be ascribed to a marked enhancement of reaction when two active reaction droplets meet during their irregular wanderings. Perhaps there is temporary growth of the active reaction zone by bubble or froth formation. As nuclei grow hemispherically, there must also be division of active droplets¹⁷ into two or more which thereafter progress independently. Unfortunately, the identification of the distribution of NO_2ClO_4 within partly decomposed crystals was impracticable as it hydrolyses so readily.

The reaction mechanism described above does not take into account the initiation of reaction in pure AP, where no source of oxidized nitrogen is available. Although we have not yet studied in detail the slow reactions occurring during the induction period, we have observed that nucleus generation often occurs within grooves on roughened surfaces (see, for example, Fig. 2c). Such surface retexturing occurs during sublimation and presumably is accompanied by other slower decomposition reactions, such as Cl_2O_7 breakdown¹⁶, capable of oxidizing ammonia. Any volatile NO_2ClO_4 produced can only participate in nucleus growth when it is effectively retained within pores and channels, progressively formed at the inner surfaces of grooves. Thus again^{17,18} we identify nuclei as specialist structures developed particularly to retain (temporarily) a fluid participant

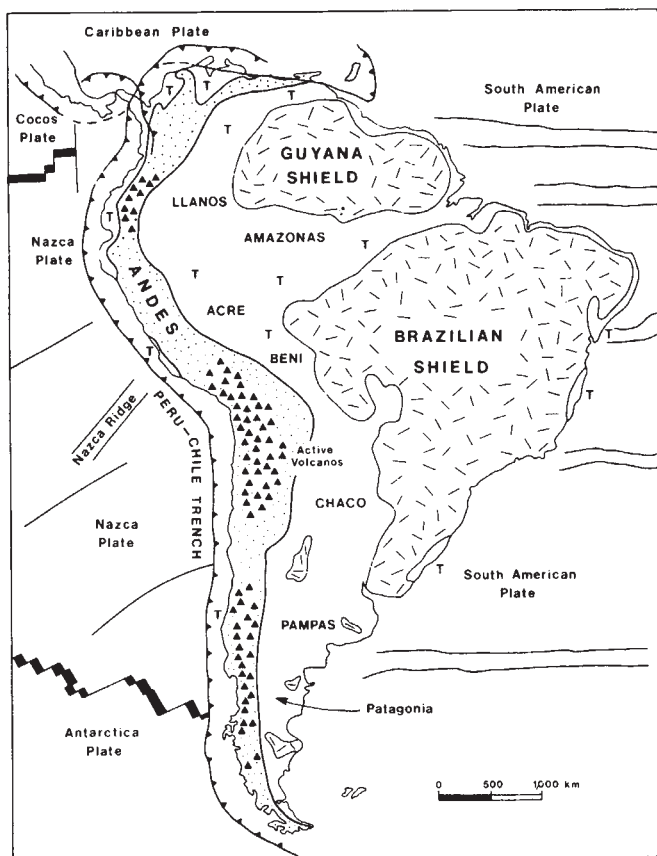


Fig. 1 Major tectonic elements of South America and adjacent ocean basins.

Present themes of the plate tectonic control on framework mineralogy come from studies of Mesozoic and Cenozoic basins²⁻⁷ supplemented by studies of modern deep sea⁸⁻¹¹ and alluvial sands¹²⁻¹⁵. More than 40 papers on the plate tectonic interpretation of sandstone mineralogy have appeared since 1970, and virtually all of these recognize three different major tectonic settings with seven to nine subdivisions using standard compositional triangles (Fig. 2).

South America, the fourth largest continent, forming 12% of all the Earth's land surface, is the most elegantly asymmetric of all the continents from a plate tectonic standpoint—the entire west coast is an active margin, all of the eastern coast from Tierra del Fuego to Trinidad is a passive margin and only its Caribbean border, where translation seems important—is moderately complex (Fig. 1). South America is also free of continental glaciation; continent-wide provenance studies of modern sands are difficult to interpret where Pleistocene continental glaciation was widespread, because it blurs the continent-wide distribution pattern of sand. The climate of South America ranges from arid, with extremely dry deserts along coastal Peru and Chile, through temperate to tropical, with up to 10 m of rainfall in northwestern Colombia. Furthermore, major climatic trends are transverse to the Andes. Physiographic contrasts are equally impressive. The Andes Mountains, the world's longest mountain range with a length of almost 9,500 km, have towering peaks, an average elevation second only to that of the Himalayas, and many high, vast, dry plateaus. Additionally, South America has sweeping plains and low plateaus—some dry and grass covered, some covered by vast jungles. Also present along much of the Brazilian coast are jungle covered mountains composed of Precambrian rocks that rise from a narrow coastal plain. The coastal physiography and climate for the entire continent is summarized in ref. 16.

Another reason to study South America is that there are data available on the petrography and chemistry of the modern river sands of the Amazon and Tocantins basins¹⁴, an area that covers

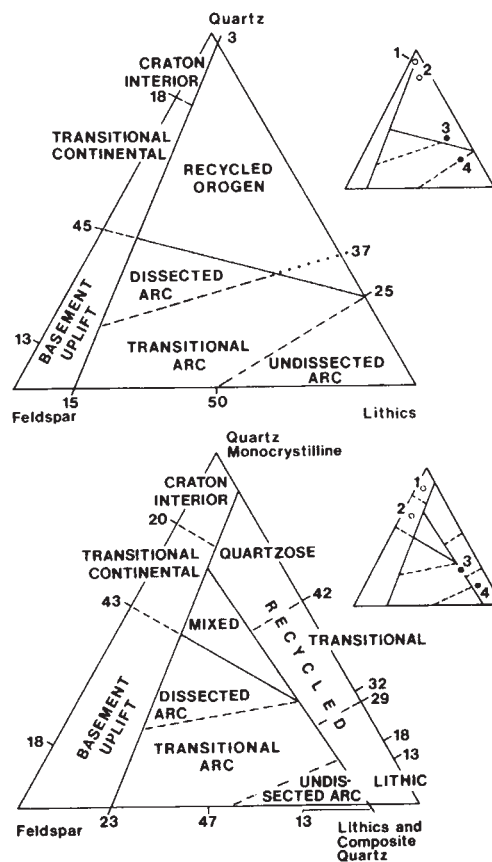


Fig. 2 Plate tectonic classification of sandstone mineralogy (after ref. 6, Fig. 1). Numbers in the small triangles indicate: 1, average composition of passive margin sands from the Rio de Plata to Trinidad; 2, beach sands from Trinidad westward along the Caribbean coast; 3, Argentine beach sands; and 4, the sands of South America's Pacific coast (see Fig. 3).

about 40% of South America plus some for the Magdalena, Orinoco, Parniba do Sul, Paraná and Sao Francisco rivers¹⁵, as well as for small rivers on the Peruvian coast. Together these river sands come from basins that drain about 65% of the continent. There are also published data on deep-sea and shelf sands of the Peru-Chile trench bordering South America¹⁷ in addition to the sands of Atlantic shelf¹⁸.

This study reports on the first-ever effort to study petrographically the sand of a single environment, the beach, around an entire continent. The technique used in sampling the beach sands of South America was based on that of Du Rietz¹⁹, who showed how the petrology and chemistry of modern Baltic beaches become more mature as littoral drift transports sand away from the southern edge of the Baltic Shield.

Why study modern beaches? The beach is the interface between marine and non-marine environments so that all marine sands have passed through it and, with recycling, perhaps the same is true for many, if not most, ancient sandstones. Because the beach environment is one of high kinetic energy, it provides a strong test of another factor: modification of framework mineralogy by abrasion. In other words, if petrologists can correctly identify the tectonic setting of a landmass from its beaches, surely they could do so from its fluvial sands. Also, the beach is an easy environment to reach and sample—an important factor when an entire continent with a coast line of 50,000 km is to be sampled. More than 200 samples of beach sand were collected in 116 field days. Beaches were selected at points of convenient access and the 27 samples reported here were selected because of their geographical representation of each major mineral association.

Where possible, fine- to medium-sand was collected to best match the dominant size classes with midpoints of 177 and 350 μ m (2.5 and 1.5 ϕ) as reported by Shea²⁰ based on his

compilation of 11,212 analyses chiefly of onshore sands and sandstones, but including some sands from modern continental shelves. These two size classes include ~35% of his present average grain-size distribution. Because grain sizes of samples in this study are coarser than those of most deep-sea sands, more rock fragments with their greater provenance information can be expected than in studies of typical fine-grained, deep-sea sands derived from the same source. To avoid the pitfalls of studying only a narrow size fraction and missing important diagnostic grains²¹, the entire sand was impregnated and studied using standard counting procedures¹⁵ after staining for potash feldspar.

Contrasts in the beach sands of South America are dramatic as judged by variations of quartz (Q), feldspar (F), and rock fragments (Rf) and dominant types of rock fragments (Fig. 3). Beach sands of South America's leading margin, the Pacific coast, have an average Q:F:Rf ratio of 19:13:68, whereas the continent's trailing margin coastline from the Rio Plata to Trinidad, a coastline bordering the Brazilian and Guyana Shields, averages 95:3:2, a staggering difference which is even more impressive when it is realized that mountains composed of Precambrian crystalline rocks border much of the South Atlantic coastline of Brazil. A petrographical count of each size fraction from a beach at Huacho in Peru and one from Natal in Brazil confirm these contrasts (Fig. 4). Downstream increase in quartz content in both the Amazon and Orinoco rivers effectively eliminates significant Andean contributions to beach sands from the mouth of the Amazon to Trinidad (Fig. 5). The rock fragments of the two major mineral associations are also totally different—volcanics predominate along most of the Pacific coast whereas rare alterites and igneous and metamorphic grains are characteristic of beach sands between Rio de la Plata and Trinidad.

Two smaller mineral associations are present and both are of great interest. All the Argentine coast from Rio de la Plata to Tierra del Fuego has an Andean, leading edge composition only slightly more mature than that of Pacific beach sands—the Q:F:Rf ratio averages 33:15:52—and again volcanics are its dominant rock fragments. Although the entire Argentine coast is a trailing margin, its beach sands have a leading edge composition because Patagonia is narrow and its bedrock near the coast is largely Tertiary molasse itself derived from the Andes. In addition, rainfall is scant, thus minimizing increase in maturity downstream from the Andes. One important implication immediately follows—use of sandstone mineralogy alone to identify palaeoplate setting can result in tectonic misclassification unless the basin's characteristics and regional setting are also considered.

The fourth and smallest association occurs from Trinidad to the mouth of the Magdalena River in Colombia. Here beach sands have an average Q:F:Rf composition of 78:5:17, although locally rock fragments are more abundant. This predominance of quartz—as along the southwestern coast of Brazil—is surprising given the proximity of metamorphic highlands close to the shoreline. Dominant rock fragments are metamorphics derived from the Venezuelan Andes.

Preliminary results based on thin section study of light minerals indicate four major mineral associations for South American beaches: two immature leading edge associations and two trailing margin association, one from the Rio de la Plata to Trinidad and the other along the Caribbean coast from Trinidad to the Magdalena River. Compositionally, west coast beaches plot on the boundary between undisectioned and transitional arc of Dickinson and others⁶ and Argentine beaches plot as a recycled orogen (back arc basin), although near the boundary to transitional arc; Venezuelan beaches plot as a recycled orogen near the boundary of the craton–interior field and beaches from the Rio de la Plata to Trinidad plot in the craton–interior field (Fig. 2, inset). Thus my preliminary results confirm the fields suggested by Dickinson and others⁶.

These preliminary results pose several interesting questions: Where a leading edge of a continent becomes narrow, its

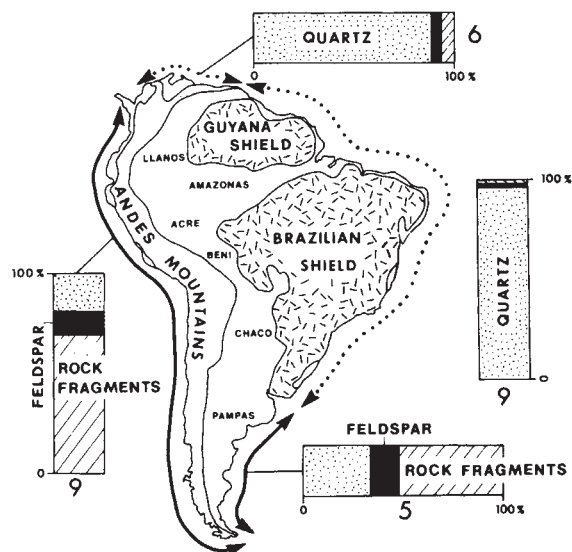


Fig. 3 Major, broad, mineral associations of South American beach sands.

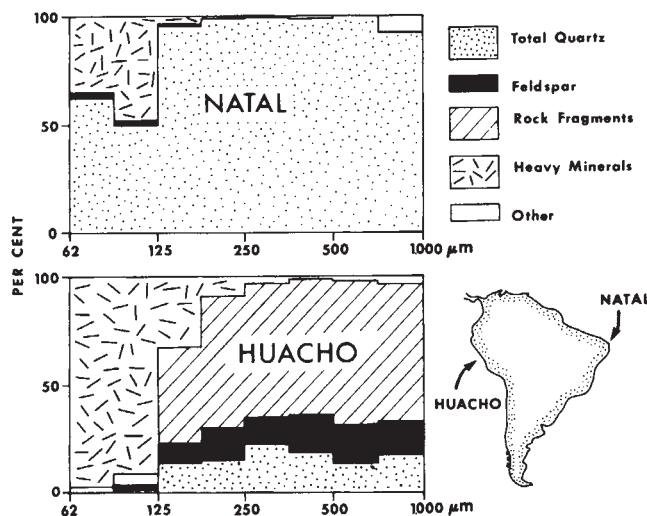


Fig. 4 Fraction analysis of beach sand at Huacho, Chancay Province, Peru and Natal, Rio Grande do Norte, Brazil shows great contrast in composition between each size grade.

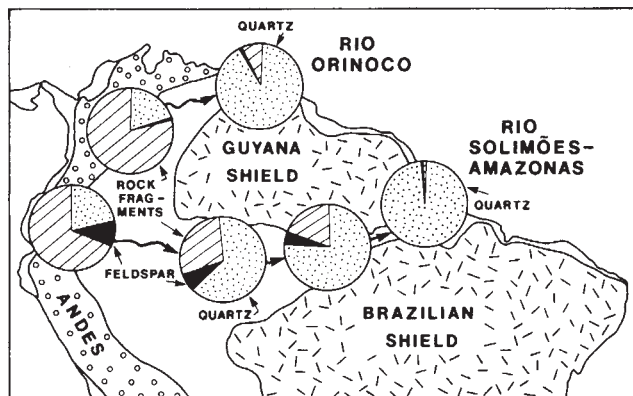


Fig. 5 Downstream increase in compositional maturity—more quartz and fewer rock fragments—in Orinoco and Solimões-Amazonas Rivers. Data for Solimões-Amazonas from ref. 14.

characteristic composition can also occur on its 'backside', passive margin, especially where a dry climate prevails, as in Patagonia in Argentina. What implications does this have for the palaeogeographic reconstructions of ancient basins? How will a wet versus dry climate on the backside of a continent affect the mineralogy of its sandy detritus reaching the sea?

Abrasion on the beaches of South America's western leading edge does not reduce our perception of the towering land mass on shore; on the other hand, the low feldspar content of beaches bordering Brazil's Serra do Mar commonly gives little hint of these Precambrian mountains. Would this be true if the coastal climate were arid rather than humid?

Why do some of the continent's larger rivers show such a great change in mineralogy from Andean source to Atlantic mouth? Would this happen if their river basins had a subarctic, temperate or arid climate?

How commonly do the boundaries of major mineral provinces along a continental margin coincide with the mouth of a large river and produce contrasts like that on opposite sides of the Rio de la Plata, whose estuary separates sand of leading edge composition in Argentina from sand of trailing margin composition in Uruguay?

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How carefully can we recognize subprovinces along the Andean leading edge, either on- or offshore, and relate them to onshore geology?

The effect of tectonic control on sandstone composition seems to be overwhelming, but how much relief is needed to overcome the effects of high rainfall? Could there be areas where we could sample a 'controlled experiment'?

As more samples are reported from modern beach sands and the adjacent offshore, how well will they agree? Will onshore sands have a different composition than their bordering shelf or deep seas?

Are we near the stage in regional studies of modern sands that we can deduce the sand composition of an entire continent? If so will computer modelling add a new dimension to provenance studies in the not too distant future?

This research was funded by NSF grant EAR-80-18209. I received much help in every coastal country of South America, which will be fully acknowledged when final results are published but, until then, I thank W. R. Dickinson (University of Arizona) L. J. Suttner and A. Basu (Indiana University), K. O. Emery (Woods Hole Oceanographic Institute) and J. Barry Maynard, (University of Cincinnati) for helpful advice.

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A new method for environmental analysis of particle size distribution data from shoreline sediments

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The study of particle size distributions is commonly used to investigate sedimentary processes and environments. Originally such work concentrated on typifying these distributions by their sample moments, while more recent statistical studies have proposed fitting parametric models to sample data and then using the resulting parameter estimates. We have investigated the use of log-hyperbolic and skew log-Laplace models to distinguish between beach and dune sands, with the aim of classifying the depositional environment of some mesolithic middens. We found that the former models were unsatisfactory for various numerical and physical reasons, while the latter provide a simple and robust method which is useful for the classification of sand sediments.

Barndorff-Nielsen^{1,2} and Bagnold and Barndorff-Nielsen^{3,4} demonstrated the statistical and geological advantages of using log-hyperbolic distributions to interpret particle size distributions. The method eliminated¹ the mathematical arbitrariness and unreliable assumption of normality associated with the use of low-order moment measures (such as, mean, median, skewness, kurtosis and developments of them⁵⁻⁷, and (2) used a mathematical distribution that had been directly related to the geological processes of transportation and deposition responsible for the sediments^{3,4}. Four parameters were utilized to describe the log-hyperbola: ϕ , γ , μ and δ (Fig. 1). Bagnold¹⁻⁴

suggested that the gradient ϕ was related to the significance in the particle size distribution of saltation, and that the gradient γ corresponded to the significance of coarser grains which 'creep'.

We have employed this statistical procedure to characterize the particle mass-size distributions with data obtained at $\frac{1}{2}\phi$ intervals obtained by standard procedures^{8,9} from coastal beach, salting and dune environments in the Inner Hebrides, Libya and South Wales; and, in particular, to identify the depositional environments, and hence the palaeogeography, represented by shell sands of unknown origins obtained in trial pits and cores beneath late mesolithic shell middens investigated on the east coast of Oronsay, Inner Hebrides¹⁰⁻¹³ (Fig. 2). In this latter study, samples were taken from along four transects crossing several depositional environments.

The fitting of the log-hyperbola was satisfactorily achieved both on our reworking of the published Bagnold and Barndorff-Nielsen^{2,3} data and on a substantial proportion of our own data¹⁴. However, in four out of our 15 attempts to use this procedure no numerically stable solution was obtained. Detailed numerical investigation of these four cases revealed that the likelihood function was virtually flat with respect to the parameter δ . The physical explanation is that there are many different log-hyperbolic distributions, differing only in curvature at the peak of the distribution, which fit the data equally well. This arises because of the coarse sequence of sieves used in the collection of our data: $\frac{1}{2}\phi$ rather than the $\frac{1}{4}\phi$ intervals used in the Bagnold and Barndorff-Nielsen data. The transition from 'fine-grade fraction' to 'coarse-grade fraction' typically occurs over a range rather less than $\frac{1}{2}\phi$. Consequently, if the peak of the distribution occurs close to a mesh size of any of the sieves, then the available data provide little information on the value of this parameter δ which essentially measures the curvature of the distribution at its peak. Use of alternative numerical techniques, in particular the programs used by Barndorff-Nielsen and his colleagues¹⁵, did not alleviate the numerical problem. Note also that the computation, using any of the numerical

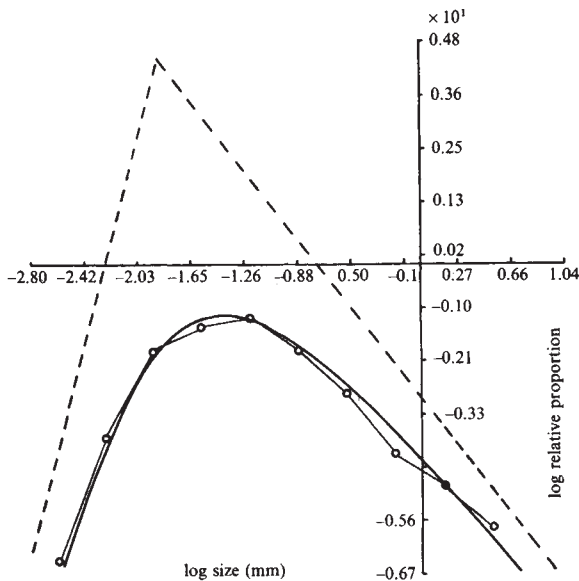


Fig. 1 Sample TUS1-6 and fitted log-hyperbolic distribution. The asymptotes to the hyperbola are shown as dashed lines. The four parameters of the distribution ϕ , γ , μ and δ are defined, respectively, as the arc-tangents of the acute angles made by the two asymptotes with the horizontal, the abscissa of the point of intersection of the asymptotes, and the difference (divided by $\sqrt{\phi\gamma}$) in ordinates of the point of intersection of the asymptotes and the maximum of the hyperbola.

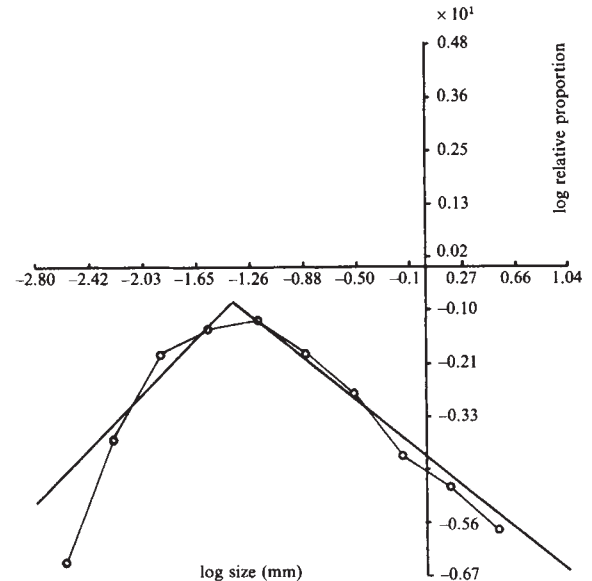


Fig. 3 Truncated skew log-Laplace distribution fitted to particle size distribution TUS1-6. The three parameters of the distribution α , β and μ are defined, respectively, as the reciprocals of the arc-tangents of the acute angles made by the two lines with the horizontal axis and the abscissa of their point of intersection.

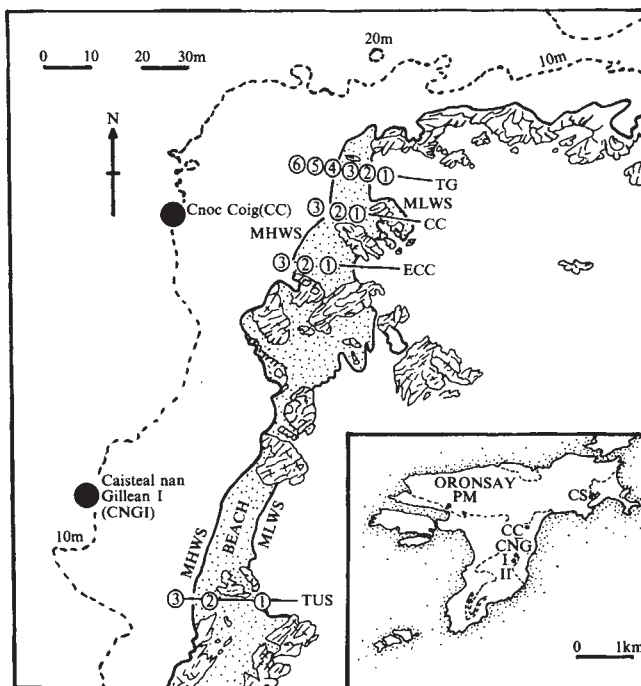


Fig. 2 Sample provenance on beach and dunes, Oronsay, Inner Hebrides and the location of the mesolithic middens and mid-Holocene shoreline (inset, after Jardine¹⁰). Middens: CC, Cnoc Coig; CNG I and II, Caisteal nan Gillean I and II; CS, Cnoc Sligeach; PM, Priory Midden.

techniques, proved prohibitively expensive for application to the large number of samples involved in our study.

Another physical explanation of our difficulties is that the sand samples we dealt with were typically less 'pure' than those described by Barndorff-Nielsen¹⁻³. Many of our samples were taken from less ephemeral locations, and many were indeed from archaeological sites, and might, therefore, have been subject to diagenetic and pedological changes.

To cope with these problems, we have fitted a distribution which is essentially described by two straight lines instead of a

hyperbola when the mass-size distribution is plotted on log-log axes (Fig. 3). Statistically this was achieved by fitting a skew log-Laplace distribution using the program LAPSKEW.FTN¹⁶. This distribution was truncated to allow for the finite number of sieves and the inevitable grouping of the distribution at the upper and lower ends. The skew log-Laplace distribution can be regarded as the limiting form of the log-hyperbolic distribution obtained by letting the parameter δ tend to zero. It is conveniently parameterized by parameters, α , β and μ . Where α and β correspond to the reciprocals of ϕ and γ , and corresponds directly to the parameter μ of the log-hyperbolic distribution. LAPSKEW.FTN utilizes a modified Davidson-Fletcher-Powell algorithm¹⁷ for determining the values of α and β for fixed μ and then a grid search for determining μ .

To illustrate the effectiveness of this approach, we have calculated and plotted values of α , β and μ for modern and mid-Holocene sand samples associated with mesolithic shell middens near the eastern shore of the Hebridean island of Oronsay (Fig. 2). Two- and three-dimensional plots of all possible combinations of the parameters have been generated, archived^{9,18}, and inspected. The equivalents of Friedman's^{5,6} plots of standard deviation against mean; skewness against mean and skewness against variance have also been plotted as $\alpha^2 + \beta^2$ against μ ; α/β against μ and α/β against $\alpha^2 + \beta^2$. Most of these plots present the same pattern of results and conclusions as for the case of α versus μ .

The plot of α versus μ , shown in Fig. 4a, reveals two clear clusters distinguished by their bay of origin. Both the mesolithic and modern samples have higher values of μ (the 'typical' grain size) at Caisteal nan Gillean (CNG) locations than at Cnoc Coig (CC) locations. Second, the values of α clearly separate modern dunes from beach sands at each location.

More detailed examination of one location—the modern environments and mesolithic sediments of CC—shown in Fig. 4b, indicate other important trends. Sediment samples from each transect CC, ECC and TG (Figs 2 and 4c) show a systematic variation in the values of α and μ from lower, via mid-beach into upper-beach and dune sands. Presumably this reflects the impact of the changing character of the transportational and depositional processes affecting the sediment.

The transects themselves are arranged in a clockwise rotation (with increasing μ and α values) from transects ECC through CC to TG (Figs 2 and 4c). This corresponds to, and is

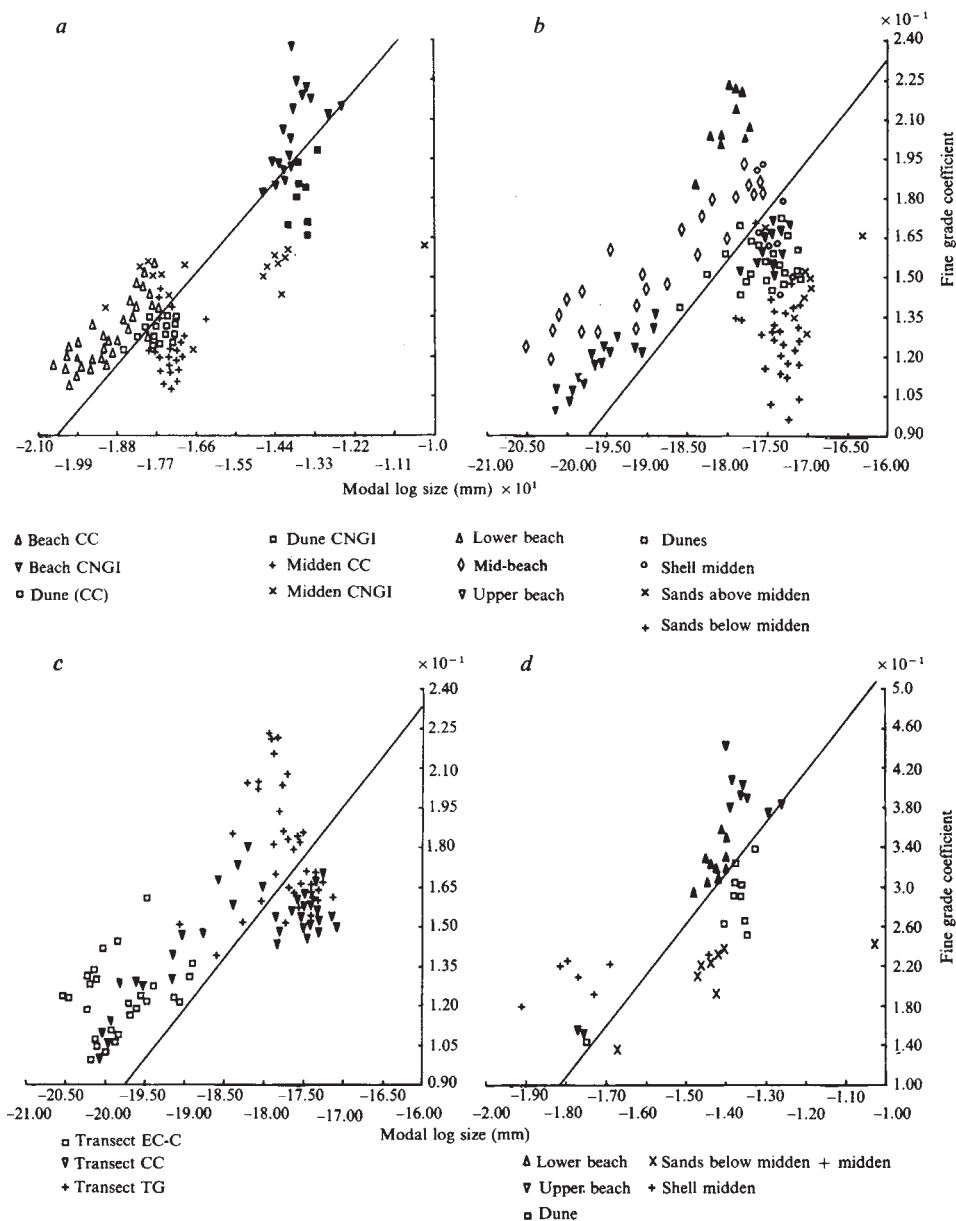


Fig. 4 Scattergrams showing the plots of modal log grain size and fine-grade coefficient of beach, dune and midden sands and the linear discriminant functions¹⁹ separating modern beach and dune sediments at: a, eastern Oronsay; b, Cnoc Coig (showing midden samples); c, Cnoc Coig (distinguishing transects); d, Caisteal nan Gilleann.

presumably the result of, a gradient from the exposed coastal locations to the relatively sheltered ECC with its protecting offshore rock bars and skerries.

In some cases, sands from the base of the dune and back of the beach are scarcely distinguishable in terms of their mass-size distribution. In practice, the components of this transitional zone are often difficult to separate in this field. The graphs of the linear discriminant functions¹⁹ separating modern beach and dune sands (Fig. 4b, c) generally corroborate and clarify these patterns.

The sands immediately above and below the mesolithic CC shell midden are principally dune sands, but some plot in a position indicating that they might represent the actual beach/dune transitions. The sands of previously unknown origins obtained by coring beneath the midden are seen to be dune sands (Fig. 4b). These data have archaeological and palaeogeographical significance, as it was anticipated that the middens had accumulated on beach sand associated with the mid-Holocene sea-level maximum¹⁰.

The sands obtained from interstices between the shells of the actual CC midden form a further distinct cluster straddling the upper beach/dune base transition. This suggests that (1) the middens were located at that position and/or (2) these particle mass-size distributions reflect sand introduced on animals, shellfish and seaweed gathered from the foreshore and/or introduced

by feet and hands after harvesting activities. The sands within the CNG I shell midden (Fig. 4d) resemble more closely deposits found on the modern mid-beach, rather than upper beach, suggesting that the second group of explanations might be more applicable in this case.

This case study has concentrated on plots of α versus μ . In other cases, our unpublished work indicates the value of inspecting three-dimensional plots of α against β against μ . Single plots of β against μ have also provided adequate environmental discrimination in other situations. The more complex studies representing the equivalents of mean, skewness, variance and so on have not yielded notable additional information.

The data indicate that fitting skew log-Laplace distributions to particle mass-size distributions can provide a sensitive tool for the characterization and discrimination of depositional process and environments in coastal locations. The method is simple and cheap in computational terms: it can even be quickly approximated 'by hand'. It is statistically robust and has been used successfully with data obtained at $\frac{1}{2}\phi$ intervals facilitating notable savings in laboratory analyses. It retains the important assets of being directly comparable with the log-hyperbolic distribution, and consequently the slope parameters of the skew log-Laplace distribution may also be directly related to geological processes. It makes no assumptions of an underlying normal

particle size distribution, but reliably describes important attributes of particle mass-size distributions.

We thank Mrs D. A. Y. Timmins for particle mass-size distribution data; Drs G. W. Jardine and P. A. Mellars for discussions; D.D.G. acknowledges the financial support for field work from the University of Sheffield research grant nos 359, 561, and 710 and W.O. acknowledges receipt of a scholarship from the Studienstiftung des deutschen Volkes.

Received 29 May; accepted 14 August 1984.

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Effect of benthic mixing on the information content of deep-sea stratigraphical signals

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Benthic mixing or bioturbation affects sediments in various ways, including: (1) the production of trace fossils, (2) mechanical and/or chemical alteration of the sediment, and (3) filtering or smearing stratigraphical signals¹. Because mixing alters both the slopes and amplitudes of any recorded events, some knowledge of the process is essential for the correct interpretation of the signals. In view of recent trends towards high-resolution stratigraphy^{2,3} and signal unmixing⁴⁻⁶, the frequency characteristics of the benthic mixing filter must be understood to determine which types of signals can be detected after mixing. Analyses of ash and tektite profiles in deep-sea cores from various geographical regions indicate that even signals from cores having sedimentation rates as high as 7 cm kyr⁻¹ will show severe attenuation of frequencies higher than 0.35 cycles kyr⁻¹ (periods shorter than 2.9 kyr) resulting in loss of ability to resolve closely-spaced events. Most signals will experience much more serious high-frequency attenuation, however. The severity of high frequency loss is directly related to sedimentation rate ($R=0.97$ for the cores examined), suggesting that this is the most important variable to take into account when considering a core for palaeoceanographic or palaeoclimatic study.

Several models have been developed to explain the filtering suffered by stratigraphical signals as they pass through the sediment mixed layer⁷⁻⁹. They express the mixing function (system impulse response) in terms of physical parameters, usually mixed-layer thickness and a diffusion coefficient, which determines the rate of transfer of material through the mixed layer. All previous workers have treated benthic mixing as a linear time-invariant system. In fact, there are several reasons why the process is expected to vary with time. Productivity, and hence

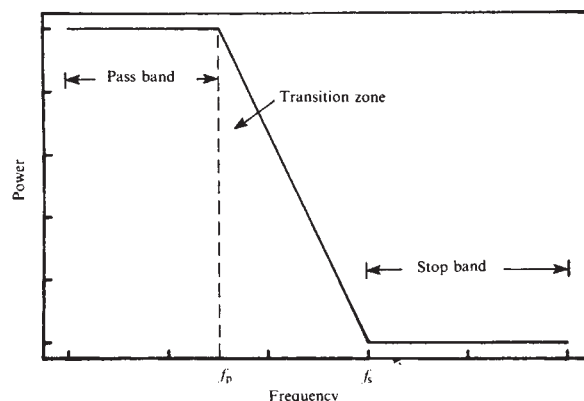


Fig. 1 Aspects of a lowpass filter. f_p and f_s are the passband and stop-band cutoff frequencies, respectively.

sedimentation rate, varies as a function of climate. The number of benthic organisms (and hence mixing intensity), should depend on the amount of organic matter reaching the sediments¹⁰. Because the time-varying aspects of the mixing system are not well characterized, however, simplification is necessary.

The output and the input of a linear time-invariant system are related by the convolution integral¹¹. A frequency response approach is generally used to identify such a system, which is accomplished by taking the Fourier transform of the convolution equation. The system is then represented as

$$G(\omega) = H(\omega)F(\omega)$$

where $G(\omega)$, $H(\omega)$, and $F(\omega)$ are, respectively, the Fourier transforms of the output (filtered) signal, the impulse response, and the input signal. $H(\omega)$ is called the system transfer function, as it completely describes input and output relationships¹². Most natural processes, including benthic mixing, involve smoothing or lowpass filters, meaning that the low frequencies are passed through, while the high frequencies are attenuated. Characteristics of a typical lowpass filter are shown in Fig. 1. The passband extends from zero frequency to the cutoff frequency f_p . The stopband extends from the cutoff frequency f_s to the Nyquist frequency. The region between f_p and f_s is the transition region.

Many volcanic ash and tektite profiles have been measured in deep-sea sediment cores from a broad range of geographical areas¹³⁻¹⁷. These profiles represent system impulse response functions due to the instantaneous nature of the inputs. Officer and Lynch¹⁸ compiled 16 of these profiles, which were then fit to theoretical mixing profiles using the model of Guinasso and Schink⁹. These profiles were thus parameterized in terms of mixed layer thickness d and diffusion coefficient D . These parameters and a truncated series solution of the mixing equation¹⁸ can be used to regenerate the mixing profiles. The regenerated curves offer an advantage over the measured profiles when investigating the mixing transfer function in that counting noise and mixing heterogeneity are effectively smoothed out. The power spectrum of each mixing function is calculated to determine the frequency characteristics of the transfer functions.

In practice, the passband cutoff frequency is usually defined as the halfpower point¹⁹, that is the frequency at which one half of the input signal power is attenuated (-3 dB). As an example, the mixing function from core RC17-126, is shown in Fig. 2. The halfpower point (-3 dB) is at a frequency 0.14 cycles kyr⁻¹ (f_p). Frequency response decreases ('rolls off') at roughly 10 dB per octave (an octave refers to a doubling of frequency). The benthic mixing filter analyses for the 16 profiles are summarized in Table 1, and -10 dB (signal attenuated one order of magnitude) points are provided to give some idea of filter roll-off. Most stratigraphical signals (such as, oxygen isotopes) do not have a signal-to-noise ratio >10 dB (ref. 20), so these numbers also give some idea of an absolute attenuation frequency (f_s).

The f_p values shed some light on the affects of benthic mixing on stratigraphical signals, as they span well over an order of

magnitude. Even in the cases of minimal signal attenuation, all wavelengths $\leq 3,000$ yr are substantially attenuated. This level of filtering will smooth out rapid transitions or transients (such as glacial-interglacial transitions), and obscure the relationships of closely-spaced events. This is true even when sedimentation rates are high (for example, 7 cm kyr^{-1} in cores V19-28 and V19-29). Most deep-sea cores used for palaeoceanographic or palaeoclimatic analysis tend to have sedimentation rates of the order of $1\text{--}2 \text{ cm kyr}^{-1}$. Mixing filters in Table 1 corresponding to cores of this type have a passband cutoff at a period of roughly $10,000\text{--}20,000$ yr. While filters of this severity would not obscure Milankovitch or orbital frequencies ($20 \text{ kyr}\text{--}100 + \text{kyr}$), they will lead to some reduction in the amplitudes of these spectral peaks²¹ and will certainly make the results of any high-resolution study (sampling every $500\text{--}1,000$ yr) questionable, unless there were some sort of signal restoration (deconvolution).

A relationship between sedimentation rate and mixed layer thickness has been observed in equatorial Pacific box cores²², suggesting a link between the amount of benthic mixing activity, primary productivity of the overlying surface waters, and the consequent flux in surface sediments. A strong correlation between sedimentation rate and mixed layer thickness would not be expected in areas with terrigenous influx, or in areas not so strongly controlled by biogenous sediment production. Using radiotracers in cores from diverse geographical areas, DeMaster and Cochran²³ found no systematic relationship between depth of mixing or mixing rate and sedimentation rate. A similar statement can be made for the cores listed in Table 1.

The values in Table 1 suggest that sedimentation rate is the variable with the most direct influence of f_p , and hence the information contained in a deep-sea core. A linear regression of f_p values against sedimentation rates for the 16 entries in Table 1 yielded the equation $f_p = 0.05 v$, with a correlation coefficient of 0.97. Rewriting in terms of period, and rearranging, yields $v(\text{cm kyr}^{-1}) = 20.0/\text{period}(\text{kyr})$. This equation, based on the half-power points from the benthic mixing filters examined, can be used to find the appropriate minimum sedimentation rate required to ensure recovery of a given signal period (or frequency). To recover a signal of period 4 kyr, for example, requires a minimum sedimentation rate of about 5 cm kyr^{-1} . Many 'high-resolution' studies hope for better resolution than this, yet rarely use cores having such high sedimentation rates. Even if the -10 dB points are used to construct an equation ($f_{-10 \text{ dB}} = 0.14 v - 0.01$; $R = 0.97$), in the hope that the 10 dB of lost gain can be restored by deconvolution, a sedimentation rate of 2 cm kyr^{-1} still yields a resolution of only ~ 4 kyr. Resolving

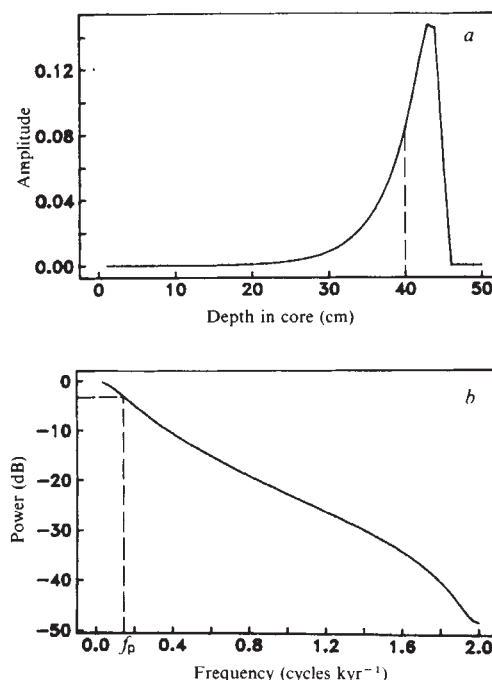


Fig. 2 Mixing function from core RC17-126. *a*, The impulse-response function, or time-domain representation of the filter, as measured from volcanic ash concentrations down core. Tracer impact depth was set arbitrarily at 40 cm (vertical dashed line). Vertical scale is in arbitrary units, and reflects unit-area normalization of the curve. *b*, The frequency-domain representation of the filter. Passband cut-off frequency (f_p) is taken to be the half-power (-3 dB) point.

a period of 1 kyr requires a core having a sedimentation rate of roughly 7 cm kyr^{-1} . That benthic mixing places severe restrictions on the time resolution of data recovered from deep-sea cores has long been appreciated²⁴, but the quantitative effects of mixing on signal recovery have received little attention. Hence, sampling of deep-sea cores often proceeds on arbitrary or intuitive grounds, usually with an optimistic idea of stratigraphical resolution. Sedimentation rate is seen to be the primary control and limiting factor on signal resolution in deep-sea cores. Unless there is some way of increasing the signal-to-noise ratio (such as signal stacking), and/or a core of extremely high sedimentation rate is used, there is little to be gained from

Table 1 Benthic mixing filter characteristics

Core	Sedimentation rate $v(\text{cm kyr}^{-1})$	Time-domain filter*		Frequency-domain filter	
		Mixed layer thickness $d(\text{cm})$	Diffusion coefficient $D(\text{cm}^2 \text{ kyr}^{-1})$	f_p (-3 dB point) (cycles kyr^{-1})	-10 dB point (cycles kyr^{-1})
V19-28	7.0	8	30	0.37	1.000
V19-29	7.0	10	20	0.33	0.83
E48-23	2.5	5	9	0.20	0.56
V27-19	2.25	8	6	0.13	0.32
RC17-126	2.0	6	8	0.14	0.37
V16-76	1.51	15	20	0.039	0.11
RC9-58	1.0	12	8	0.037	0.095
V19-153	0.75	13	3	0.031	0.068
V19-297	0.7	16	40	0.015	0.046
RC9-137	0.64	16	8	0.016	0.046
RC9-143	0.51	18	6	0.011	0.033
V29-39 (ash)	0.5	7	1	0.036	0.083
V29-39 (tektite)	0.5	8	2	0.027	0.072
V29-40 (ash)	0.5	12	5	0.017	0.047
V29-40 (tektite)	0.5	14	4	0.016	0.041
V16-70	0.22	13	2	0.007	0.019

* The values in these columns are from ref. 18. Approximation values rather than optimization values were used since approximation equations were employed to reconstruct the impulse responses.

very closely-spaced samples in constructing downcore stratigraphies.

My work on benthic mixing has been supported by the US Office of Naval Research (contract no. N00014-80-C-0440). I thank W. H. Berger and H. R. Thierstein for reviewing the manuscript and for helpful suggestions.

Received 9 March; accepted 2 August 1984.

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Pre-Columbian age for North American *Corispermum* L. (Chenopodiaceae) confirmed by accelerator radiocarbon dating

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Criteria for recognizing alien (historically introduced) plants in continental floras are largely inferential, involving range disjunctions, absence in the first botanical records for a region, pioneer status in secondary successions ('weedy habit'), and, to a lesser extent, the fossil record. Decisions on whether a species is native or introduced commonly appear in local and regional floras. In a few cases, the plant's occurrence in the fossil record has reversed its presumed alien status¹. Ironically, the appearance of assumed introduced species in prehistoric contexts could also be taken as an index of modern contamination due to stratigraphical mixing. We now present conclusive evidence for the prehistoric occurrence of *Corispermum* L. (Chenopodiaceae), a herbaceous annual growing on sandy soils throughout western North America, where it was previously reported as adventive from Eurasia. The use of tandem accelerator mass spectrometry (TAMS) for direct radiocarbon measurement of seeds eliminates problems with stratigraphical mixing and using this method we verify a pre-Columbian age for *Corispermum*.

The genus *Corispermum* is best represented in the semi-deserts of Eurasia. *Corispermum hyssopifolium* L. and *C. nitidum* Kit ex. Schult. are the two Eurasian species that today occur in North America, thought to have been introduced at the time of European settlement. *Corispermum hyssopifolium* has been regarded as endemic to southern Russia and adventive or partly indigenous in central and southern Europe. It is the most widely distributed species in North America. *Corispermum hyssopifolium* and *C. nitidum* are the only species reported in modern floras of Alaska and the Yukon, but at least five species

with no recognized Old World synonyms have been described from type specimens in the Rocky Mountain states, US. Three of these species, *C. villosum* Rydb., *C. emarginatum* Rydb., and *C. marginale* Rydb., were described at the turn of the century by Rydberg². His reputation for taxonomic splitting³ may have influenced some workers in synonymizing his and other North American species with *C. hyssopifolium* or *C. nitidum*^{4,5}. Further confusion results from at least one worker assigning alien status to one of the North American species (*C. villosum*) which Rydberg described⁶.

The suggestion in many North American floras that *C. hyssopifolium* arrived only recently seems peculiar in view of early historical records from what was then remote wilderness. John Richardson found it on the shores of the Great Slave Lake, western Canada in 1821⁷. In the 1870s, Joseph T. Rothrock collected *C. hyssopifolium* in Colorado⁸. Hence, it is not surprising that an extensive record for pre-Columbian *Corispermum* has emerged in areas where early botanists first collected it.

Table 1 summarizes select localities and direct or associated conventional dates for *Corispermum* seeds in fossil contexts from northwest Canada, Alaska, and the Colorado Plateaus in the United States. The seeds are very distinctive and may resemble ticks to the unaccustomed eye (*Cori* = bug, Greek; *spermum* = seed, Latin). Seeds are oval in outline, flat and slightly concave-convex, with a persistent encircling wing. The base of the wing is emarginate or rounded, and the apical end bears two minute, toothlike style bases⁹ (Fig. 1). Average seed dimensions are 5 × 4 × 0.5 mm. An attempt to distinguish species of *Corispermum* based on seed size and morphology from modern voucher specimens yielded erratic results, though in some cases fossil and modern seeds of a particular species are close facsimiles (Fig. 1c and d).

Fossil contexts for *Corispermum* seeds include alluvium^{10,11}, cave fill^{12,13}, human and herbivore coprolites^{12,14}, archaeological sites^{12,13,15–17}, and fossil packrat (*Neotoma*) middens^{18–20}. Direct evidence for *Corispermum*'s role in the prehistoric human diet include its presence in human coprolites, as parched seeds in hearths, and some 80 cm³ of seeds cached in a Mimbres pottery jar from Swartz Ruin, New Mexico. Associated ages in the various contexts range from >38,000 yr BP in the Yukon to <1,000 yr BP in archaeological sites on the Colorado Plateaus. Four of the localities contained sufficient seeds from a single context to allow direct radiocarbon measurements by TAMS. The mass of the seeds in these samples ranged from 5 to 17 mg per sample (8–15 seeds per sample). We followed routine radiocarbon dating procedures, by pretreating each sample with 1 M HCl, then 2% NaOH, again with HCl, and finally washing it thoroughly with distilled water. Target preparation involved first, pyrolysis *in vacuo*, then heating the carbonized material with powdered iron under argon to form a spheroid of cast iron. Details of target preparation and radiocarbon measurement procedures are given elsewhere²¹. All four of the direct dates generally agree with the associated ages of the samples. The oldest direct dates for *Corispermum* are 9,970 ± 460 yr BP for the Canadian and Alaskan sites and 14,500 ± 600 yr BP for the Colorado Plateaus.

A pre-Columbian age for *Corispermum* and the present discontinuity in its range between Siberia and Alaska raise questions about points of origin, dispersal routes, arrival times, and subsequent evolution in North America. In the late Cenozoic, the most reasonable dispersal route would have been from northeastern Siberia across the Bering Land Bridge to Alaska. The presence of *Corispermum* seeds in herbivore dung, tentatively assigned to *Mammuthus*, from Cowboy Cave¹² and Bechan Cave¹⁴ in southeastern Utah, suggests one mode of dispersal. In the Yukon, associated dates from alluvium containing *Corispermum* seeds indicate an arrival time >38,000 yr BP. The best candidates for the Yukon and Alaskan records are probably *C. hyssopifolium* and *C. nitidum*, the only species presently in the region and the ones that were assumed to be recent European introductions. Beringian phytogeographers, taxonomists, and palaeoecologists disagree about the timing for intermingling of

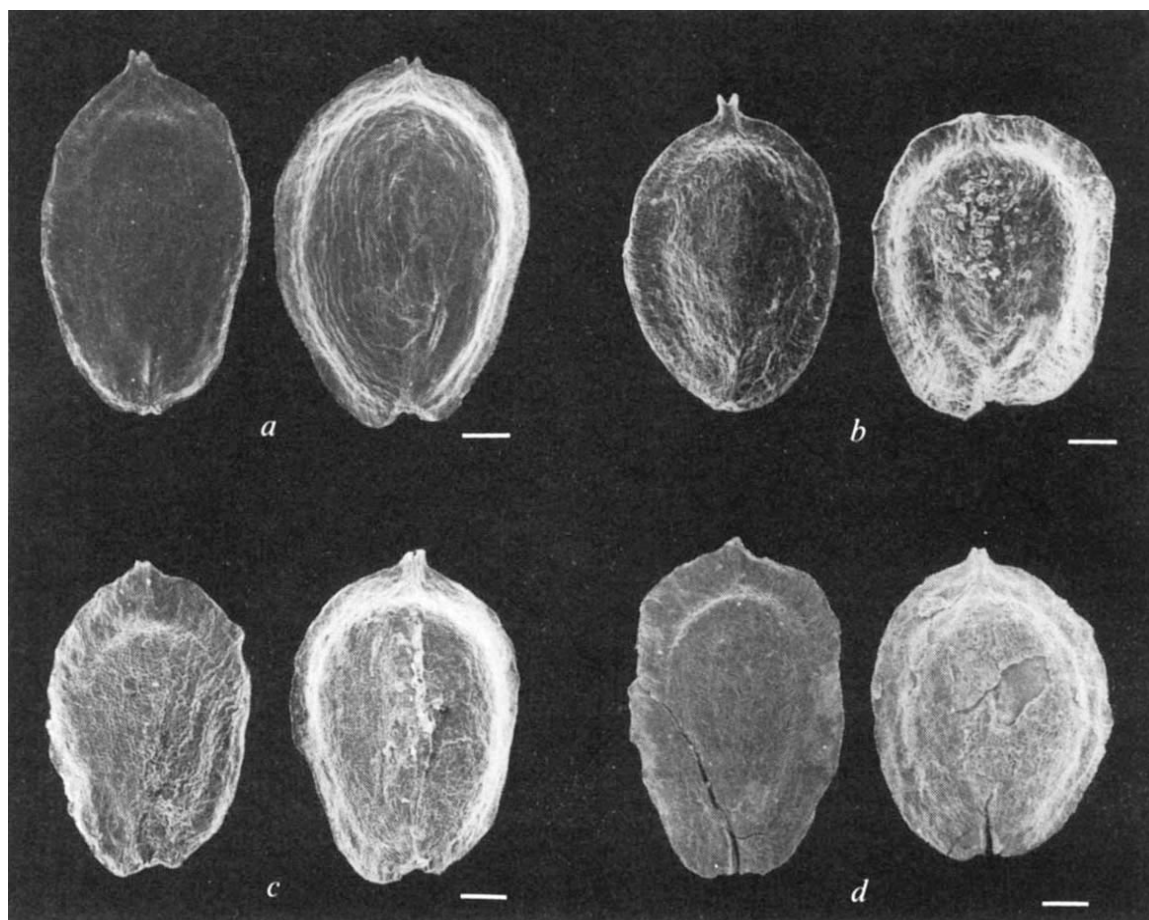


Fig. 1 Scanning electron micrographs of modern (*a-c*) and fossil (*d*) *Corispermum* seeds. Convex surfaces are shown on the left and concave ones on the right for each pair of seeds. Scale bars, 500 μm . *a*, Modern seeds of *C. hyssopifolium*; *b*, modern seeds of *C. nitidum*; *c*, modern seeds of *C. villosum*; *d*, fossil seeds from *Neotoma* midden in northwestern New Mexico. Seeds from this midden were directly dated by accelerator mass spectrometry to $3,880 \pm 240$ yr BP. Note the similarity between *d* and *c*, a North American species with no Eurasian synonym.

Table 1 Select fossil localities for *Corispermum* in North America

Area	Site	Lat.	Long.	Context	Associated age (yr BP)	Direct age (yr BP)	Ref. for associated age
Yukon Territory, Canada	Bell Basin, Upper Porcupine River	66°56.7' N	137°42.5' W	Alluvium	>38,000 (GSC-2274)	None	10
Northwest Territory, Canada	Great Bear River	64°56.5' N	125°29.75' W	Alluvium	10,600 $\pm$ 260 (GSC-2328)	9,970 $\pm$ 460 (A-3748)	10
Arctic Coastal Plain, Alaska, USA	Ikpikpuk River	69°43.0' N	154°53.0' W	Alluvium	9,670 $\pm$ 130 (I-11,073) 9,430 $\pm$ 160 (I-13,174)	9,100 $\pm$ 350 (A-3900)	11
Northern Arizona, USA	Grand Canyon, Shinumo no. 1	36°00.0' N	112°29.0' W	<i>Neotoma</i> midden	13,660 $\pm$ 160 (A-1321) 12,070 $\pm$ 600 (A-1444)	14,500 $\pm$ 600 (A-3539)	18
Northwestern New Mexico, USA	Chaco Canyon, Gallo Wash no. 2	36°00.0' N	108°00.0' W	<i>Neotoma</i> midden	4,480 $\pm$ 90 (A-1833)	3,880 $\pm$ 240 (A-3364)	20
Southeastern Utah, USA	Cowboy Cave	38°19.0' N	110°12.0' W	Cave fill, human and herbivore coprolites	13,040 $\pm$ 440 (A-1654) through 1,495 $\pm$ 60 (SI-2425)	None	12
Southeastern Utah, USA	Bechan Cave	37°40.0' N	110°45.0' W	Herbivore coprolites	>11,670 $\pm$ 300 (A-3212)	None	14

Associated ages are based on conventional radiocarbon dating of other organic material in same context as *Corispermum*. Direct ages were obtained by TAMS analyses of *Corispermum* seeds. All dates are in radiocarbon years before present. Radiocarbon laboratory numbers are enclosed in parentheses.

eastern and western steppe floras²². Because *Corispermum* may have undergone adaptive radiation and speciation after its arrival, perhaps within the past 2 Myr, its taxonomic and evolutionary status in North America should be critically re-examined.

Finally, plants of questionable origin are often herbaceous and thus contribute little biomass in depositional environments. More often than not, there is insufficient material (<1 g of carbon) for conventional β -counting methods. Hence, we anticipate application of direct radiocarbon determinations by TAMS, useful for samples of just a few milligrams, as a routine investiga-

tive tool to resolve questions about alien or native status for other problematical plants in the fossil record.

This work was funded in part by National Geographic Society grant 2410-81. The NSF Accelerator Facility for Radioisotope Analysis at the University of Arizona is supported by NSF Regional Instrumentation grant CHE 78-118576. We thank J. Matthews, R. Nelson, V. Bohrer, M. Toll, T. R. Van Devender, W. G. Spaulding, and O. K. Davis for loan of specimens or information about *Corispermum*, H. Hull for taking the scanning electron micrographs, T. Linick and L. Warneke for analytical support, and R. M. Turner and O. K. Davis for helpful reviews.

Received 19 June; accepted 13 August 1984.

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Seabird colony distributions suggest competition for food supplies during the breeding season

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There is long-standing disagreement over the way in which seabird populations are regulated¹. They may be regulated by density-dependent mortality during winter as a consequence of food shortage²⁻⁴, shortage of nest sites⁵⁻⁷, social factors⁸, or not regulated at all⁹. Ashmole^{10,11} argued that for tropical seabirds, density-dependent mortality of adults outside the breeding season is most unlikely because intraspecific competition for food close to the colony would result in prey depletion, causing adults to travel increasing distances for food as colony size increased. Breeding numbers could be regulated through a density-dependent reduction in reproductive output resulting from reduced rates of provisioning of chicks. This mechanism may not apply to seabird colonies in higher latitudes, however, because seasonal production might provide superabundant food supplies during the breeding season. Recent studies of the quantity of food consumed by breeding seabirds¹²⁻¹⁵ suggest that prey depletion also occurs in temperate regions and invites a critical test of Ashmole's hypothesis. We present here an analysis of interactions between spatial distribution and size of seabird breeding colonies and provide support for Ashmole's suggestion that seabird numbers may be limited by intraspecific competition for food around colonies during the breeding season.

We tested the idea of prey depletion by considering spatial distribution and sizes of seabird colonies, as a direct demonstration would require an expensive marine sampling programme. If Ashmole's model is correct, then in an area of uniform mean productivity, colony size should be strongly and negatively related to the number of competitors from other colonies which are feeding within the foraging range used by members of the colony. Such a relationship would not be predicted by any of

Table 1 Correlations between seabird colony size and numbers at other colonies within a given range

Species	Range (km)	Mean no. of colonies within range	Correlation	P
Gannet (12 colonies)	100	0.8	-0.92	<0.001
	150	0.8	-0.92	<0.001
	200	1.0	-0.47	NS
	300	1.8	-0.51	NS
	50	0.7	-0.56	<0.05
Puffin (15 colonies)	100	2.5	-0.81	<0.001
	150	4.9	-0.88	<0.001
	200	6.6	-0.74	<0.001
	250	9.0	-0.58	<0.05
	30	2.0	-0.45	<0.05
Shag (27 colonies)	30	3.5	-0.54	<0.01
	40	4.9	-0.48	<0.05
	50	6.4	-0.58	<0.01
	60	7.2	-0.66	<0.001
	70	8.1	-0.32	NS
Kittiwake (22 colonies)	80	9.3	-0.24	NS
	20	1.5	-0.55	<0.01
	30	3.3	-0.49	<0.05
	40	4.9	-0.67	<0.001
	60	8.8	-0.36	NS
	80	11.7	-0.32	NS
	100	14.5	-0.08	NS

Competition for food is a function of the density of conspecifics, which is proportional to the square root of the number of birds, so we have used square root transformations of numbers. NS, not significant.

the other models. Indeed, if numbers were limited by nest sites, one might expect large colonies to be spatially aggregated (the opposite trend), as geologically similar islands will tend to occur together. Further, there is no reason to expect that any pattern in the distribution of islands should affect all four data sets.

Reliable census data for all colonies of a species in a restricted area of relatively uniform oceanography and productivity are scarce. We have analysed four sets; for gannets (*Sula bassana* (L.)), puffins (*Fratercula arctica* (L.)), shags (*Phalacrocorax aristotelis* (L.)) and kittiwakes (*Rissa tridactyla* (L.)) in parts of Britain and Ireland. To restrict our analysis to areas of relatively uniform oceanography, we considered the smallest possible

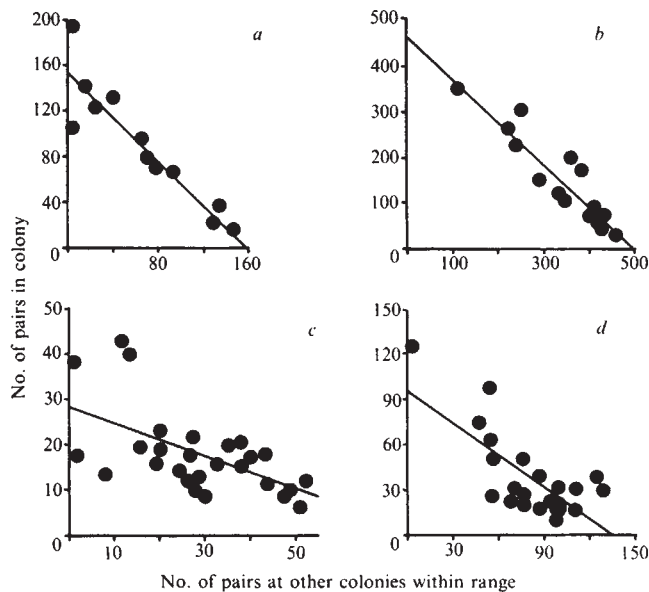


Fig. 1 Relationships between sizes of colonies and numbers at other colonies within a specific shortest sea distance for gannets, 100 km (a); puffins, 150 km (b); shags, 30 km (c); and kittiwakes, 40 km (d).

geographical area compatible with the need for an adequate sample size. For gannets, we used the most recent census data¹⁶⁻¹⁸ for each colony founded before 1960 in Scotland, Ireland and Wales. We did not include colonies in England, France or the Faeroes; the first two are in an oceanographically different water mass¹⁹, and the last is the only colony which is still heavily exploited by man as well as being distant from the colonies under consideration. We also ignored the four colonies founded in the 1970s because these are too young to have increased beyond a very small number of pairs and so are likely to be well below their optimum numbers. There is a high negative correlation for ranges of 100 km and 150 km. For greater ranges the correlation tends towards zero (Table 1). The highest correlation occurs where most colonies have only one other colony within their range and this leads to statistical difficulties of interpretation because points are not independent. This problem was overcome by carrying out 999 randomizations of colony sizes. None resulted in a correlation greater than that observed.

For puffins we took census data directly²⁰ and considered all colonies of over 1,000 pairs in Scotland north of 57°30'N, a total of 15 colonies holding over 97% of the Scottish puffin population²⁰. There is a high negative correlation for ranges of 100–200 km (Table 1).

For shags we included all colonies of 40 pairs or more in Orkney and Shetland²¹ (>95% of the Orkney and Shetland total²¹) and for kittiwakes we examined 1981 census data for Shetland²². Patterns for these species (Table 1) are similar to those already described. Species with shorter foraging ranges are more likely to be influenced by local variations in marine productivity; our assumption that mean productivity is uniform is a weakness of this test of Ashmole's hypothesis, but the fact that strong correlations do exist in spite of this strengthens our confidence in the result.

For puffins, and to a lesser extent for shags and kittiwakes, correlations over the shortest ranges are poorer than those over slightly greater ranges, partly due to the small number of colonies within the shortest range (Table 1). The tendency also arises because for short ranges the size of a colony may be influenced by the presence of a large colony just outside the range considered, whereas for large ranges the additional influence of a colony outside the range is very much less.

The strong negative relationships imply that seabird colony sizes are strongly influenced by numbers of conspecifics compet-

ing for food near to the colony. This interpretation is strengthened by the fact that the relationships are most pronounced over distances which match the foraging range of the species. Normal maximum foraging ranges of shags are 10–30 km^{23,24}, for kittiwakes 40–60 km²⁴, for puffins 10–150 km^{24,25} and for gannets 100–150 km¹⁶, while the closest relationships occur respectively over ranges of 30–60 km, 20–40 km, 100–200 km and 100–150 km (Fig. 1).

Numbers of nest sites may restrict the size of some particular colonies, but the distribution and size of other colonies usually compensates for this to raise the overall species' density to that limit set by food availability during the breeding season. The mechanism for the regulation of colony size is presumably through variations in recruitment in relation to colony breeding performance, possibly resulting from the tendency of young to recruit into their natal colony; however, recruitment processes in seabirds are poorly understood.

Received 15 June; accepted 20 August 1984.

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Excitatory amino acids directly depolarize rat brain astrocytes in primary culture

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L-glutamic acid (L-Glu) and L-aspartic acid (L-Asp) are considered to be major excitatory amino acid transmitters, causing depolarization and excitation of neurones in the mammalian central nervous system (CNS)^{1,2}. These responses have been thought to be an exclusively neuronal property as the excitatory amino acids either did not affect the potential of electrophysiologically unresponsive glial cells^{3,4}, or when an effect was seen, it was attributed to changes in external [K⁺] (refs 5, 6). Here we report that L-Glu directly depolarizes immunocytochemically-identified astrocytes in primary culture. L- or D-Asp and kainic acid (KA) also depolarized these cells while none or minimal changes in the resting membrane potentials were found in response to N-methyl-D-aspartate, D-glutamate, taurine, L-glutamine or to the inhibitory amino acids γ -aminobutyric acid (GABA) and glycine. We conclude that the membrane potential of astrocytes can no longer be thought of as being responsive only to K⁺ and that the electrophysiological effects of excitatory amino acids *in situ* may not be exclusively a neuronal property.

When astrocytes were bathed in a solution of L-Glu (10⁻⁴ M), rapid depolarization was detected, followed by a slow repolariz-

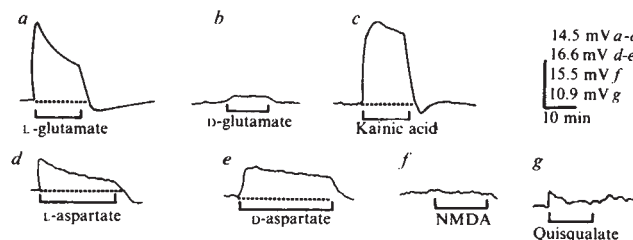


Fig. 1 *a-g*, Membrane potential responses of cultured astrocytes to excitatory amino acids and other agonists. NMDA, *N*-methyl-D-aspartate. Final concentrations of all additions were 10^{-4} M and perfused for the times marked on the figures. Dashed lines indicate pre-addition baseline. The temperature ranged between 35–37 °C and was maintained by heating the room. Membrane potentials of the cells shown before addition of the amino acids were *a*, –85; *b*, –87; *c*, –78; *d*, –56; *e*, –70; *f*, –86; *g*, –64 mV. Recordings shown in *a-g* are from separate cells. All cells responded to L-Glu (10^{-4} M).

Methods: Astrocytes were grown as primary cultures from neonatal rat brains as described previously^{10,11,15,28}, with the exception that the brain tissue was dissociated using a neutral protease (Dispase II, Boehringer-Mannheim)²⁹. These cultures show $\geq 90\%$ staining of the cells^{10,28,29} for the astrocyte marker GFAP^{8,9}. Membrane potentials were measured using standard electrophysiological recording techniques as previously described^{11,15}. Microelectrodes were filled with 0.5 M K_2SO_4 , buffered with 5 mM HEPES, pH 7.2 instead of KCl-filled electrodes as we have found that the use of K_2SO_4 -filled microelectrodes allowed us to record from the cells for longer periods of time. The bathing medium consisted of 122 mM NaCl, 2.8 mM KCl, 1.2 mM KH_2PO_4 , 1.3 mM $CaCl_2$, 0.4 mM $MgSO_4$, 10 mM D(+) glucose and 25 mM HEPES adjusted to pH 7.4 with NaOH (total $[Na^+]$ was 140 mM and total $[K^+]$ was 4.5 mM). The electrophysiological recordings were made under conditions of continuous perfusion using a Nikon Diaphot inverted microscope equipped with Hoffman modulation contrast optics as previously described¹¹. The bathing medium and stock solutions (10 mM) of the amino acids were made in glass distilled water 1 to 2 h before use. L-glutamic acid hydrochloride was obtained from Sigma Chemical Co., St Louis, Missouri.

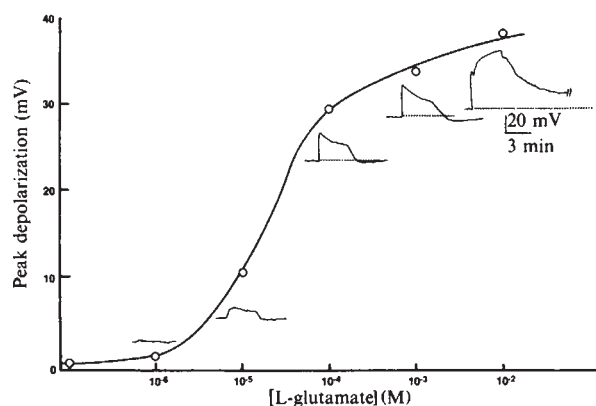


Fig. 2 Depolarizing responses to varying concentrations of L-glutamate. Initial peak depolarizations are plotted. Representative examples of the responses obtained at different concentrations are shown. Records are from a single cell. Other conditions are as in Fig. 1.

ation in the continued presence of L-Glu (Fig. 1*a*). The removal of L-Glu resulted in a rapid repolarization which was followed by a transient hyperpolarization and then a slower recovery to the baseline membrane potential (Fig. 1*a*). The mean magnitudes of the L-Glu (10^{-4} M) induced depolarization, repolarization and hyperpolarization were 19 ± 7 mV (mean $\pm$ s.d., 48 cells), 8 ± 4 mV (48 cells) and 4 ± 3 mV (46 cells) respectively. The resting membrane potentials were -71 ± 10 mV (48 cells). We have observed an L-Glu induced depolarization in all cells (48 in total) tested from six different primary cell cultures. The age of these cultures ranged from 21 to 36 days. Perfusion of D-Glu (10^{-4} M, 4 cells) resulted in only a small depolarization (Fig. 1*b*) showing that the L-Glu-induced depolarization was

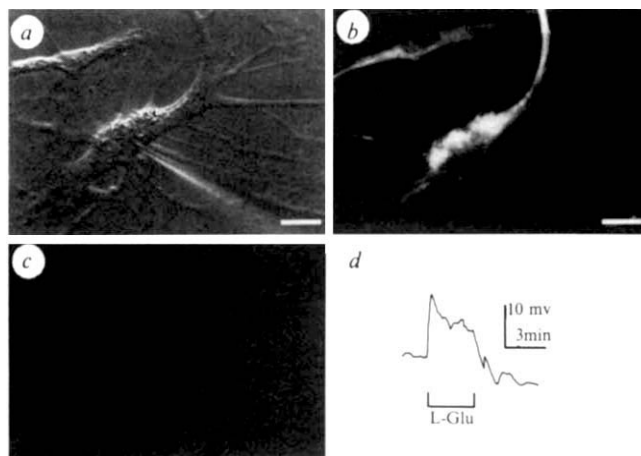


Fig. 3 Cells that are depolarized by L-Glu contain glial fibrillary acidic protein. *a*, Impaled cell viewed through Hoffman modulation contrast. *b*, Same cell after fixation and staining for GFAP, viewed through fluorescent optics. Filamentous staining is clearly visible in processes. Bar in *a* and *b* represents 20 μ m. *c*, Fluorescent micrograph of another coverslip where the primary antibody was absorbed with bovine GFAP. *d*, L-Glu (10^{-4} M) induced depolarization from same cell as in *a* and *b*. Resting membrane potential was –42 mV. **Methods:** Cells were recorded from as described in Fig. 1. The impaled cells were then photographed, an area on the underside of the coverslip was marked with a diamond object marker (Leitz) and photographs of the mark were made¹¹. This procedure allowed us to identify the impaled cell after staining for GFAP. After all recordings, photographs and marks were made the coverslip was removed and the cells stained for GFAP as described previously^{11,28}. A monoclonal antibody (Amersham) directed against GFAP was used as the primary antibody. It was specified as being positive only for glial cells in human and rat brain and did not react with a variety of other tissues. The second antibody was goat anti-mouse IgG conjugated with fluorescein. Using a Nikon Labophot microscope the areas containing the impaled cells were identified by means of the marks previously made with the diamond object marker and the impaled cells identified using the photographs. Fluorescent micrographs were then taken of these cells. Controls were established by omitting the primary antibody, or by absorbing the primary antibody with bovine GFAP and were all negative (*c*). Also, neuronal primary cultures from rat hippocampus³⁰ did not stain using this monoclonal antibody.

stereospecific. Bathing the cells with KA (10^{-4} M, 7 cells), L-Asp (10^{-4} M, 3 cells) or D-Asp (10^{-4} M, 2 cells) also resulted in a depolarization of the resting membrane potential (Fig. 1*c, d* and *e*). The magnitude of the KA-induced depolarization was usually equivalent to that seen for L-Glu whereas the effect of L- or D-Asp was generally less. *N*-methyl-D-aspartate (NMDA) (10^{-4} M, 3 cells) and quisqualate (QA) (10^{-4} M, 5 cells) were without effect, or only minimally effective (≤ 4 mV) (Figs. 1*f, g*). The inhibitory amino acids, glycine (10^{-4} M, 10 cells), GABA (10^{-4} M, 4 cells), taurine (10^{-4} M, 3 cells) and the L-glutamate metabolite L-glutamine (10^{-4} M, 3 cells) had no effect on the membrane potentials (data not shown). The lack of effect of GABA may not be surprising as only a non-neuronal low affinity [3H]-flunitrazepam binding site has been found in cultured glial cell membranes⁷.

Figure 2 shows the responses from a single cell and demonstrates that the 50% effective concentration (EC_{50}) of the L-Glu-induced depolarization was approximately 50 μ M. The shape of the response was altered at the highest concentration tested (10^{-2} M), with the appearance of a second slower depolarization that overcame the normal pattern of partial repolarization in the presence of L-Glu and a slower repolarization after removal of L-Glu (compare responses to 10^{-2} M L-Glu with 10^{-4} M L-Glu in Fig. 2). Similar results were obtained from another cell.

Since at least 90% of the cells in our cultures stain for glial fibrillary acidic protein (GFAP), and intermediate filament

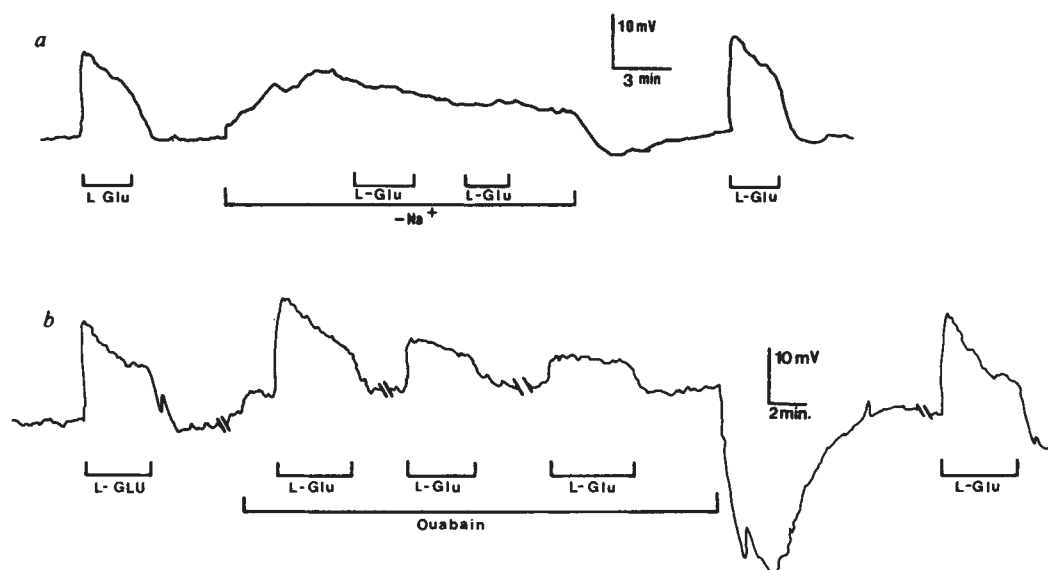


Fig. 4 The L-Glu induced depolarization requires external Na^+ . [L-Glu] was 10^{-4} M. *a*, L-Glu response in normal Na^+ medium (see Fig. 1) and in the absence of Na^+ ($-\text{Na}^+$). In the latter case, Na^+ was replaced mole for mole by choline. In the absence of impalement removal of Na^+ either did not alter the electrode potential or at most changed it by 1–2 mV. Resting membrane potential of this cell was -72 mV. *b*, Effect of 1 mM ouabain on the L-Glu induced depolarization. The breaks in the tracings represent approximately 2 min. Resting membrane potential for this cells was -70 mV.

protein considered specific for astrocytes^{8,9}, and since the cells have essentially linear current-voltage characteristics^{10,11}, we considered it unlikely that all our electrophysiological recordings were from any contaminating neuronal cells. However, because it is well-established that L-Glu depolarizes and excites neurones and as previous investigators had found no evidence for a direct electrophysiological effect of L-Glu on mammalian glial cells *in vivo*³ or *in vitro*^{4–6}, we determined whether the cells in our cultures depolarized by L-Glu were astrocytes by post-staining such cells for GFAP. Figure 3 shows a cell depolarized by L-Glu and showing positive staining for GFAP. Similar results were found in 2 other cells. Therefore, we conclude that astrocytes in our primary cultures are directly depolarized by L-Glu.

Astrocytes in primary culture show Na^+ -dependent high affinity uptake of L-Glu^{12,13}. Thus, an electrogenic uptake of L-Glu with Na^+ might be partially or wholly responsible for the depolarization. Alternatively, the L-Glu induced response could be the result of an increased Na^+ conductance. To test these alternatives we investigated the effect of removing external Na^+ on the L-Glu-induced response. Removal of Na^+ (replaced with choline) alone resulted in a depolarization, initially of 14 mV which then repolarized by 6 mV (see Fig. 4*a*). We have previously reported^{11,14} a depolarizing response to omission of external Na^+ in primary astrocyte cultures which we suggested could be partly due to inhibition of an electrogenic Na^+/K^+ pump and/or a secondary consequence of other effects of no external Na^+ , such as modification of intracellular pH due to inhibition of Na^+/H^+ exchange. In the absence of external Na^+ , perfusion of L-Glu had no further effect on the resting membrane potential (Fig. 4*a*). Subsequent addition of Na^+ and re-perfusion of L-Glu resulted in a normal L-Glu-induced depolarization identical to that seen before removal of external Na^+ (Fig. 4*a*). Similar results were obtained when Na^+ was replaced with arginine (data not shown). These results show that external Na^+ is required for the L-Glu induced depolarization. Because we found that the L-Glu-induced depolarization is not affected by tetrodotoxin (10^{-6} M) (data not shown) an involvement of veratridine-induced Na^+ channels that we have recently observed in these cells¹¹, is unlikely.

Although the present data do not allow us to distinguish between an electrogenic Na^+ -dependent L-Glu uptake mechanism, or activation of an L-Glu receptor resulting in a depolarizing conductance change to Na^+ , both mechanisms should involve an inward movement of Na^+ . Thus, the repolarization in the continued presence of L-Glu could involve activation of an electrogenic Na^+/K^+ ATPase due to an increase in $[\text{Na}^+]_i$. To test for this possibility, we studied the effect of ouabain on the L-Glu-induced response (Fig. 4*b*). Perfusion of L-Glu immedi-

ately after addition of ouabain showed no immediate change in the L-Glu induced depolarization and repolarization (Fig. 4*b*). With continued exposure to ouabain, however, there was a progressive decrease in the size of the initial depolarization due to L-Glu and the subsequent repolarization. This is likely to be a result of the gradual dissipation of the Na^+ gradient that occurs in these cells in response to ouabain¹⁵. Thus, the repolarization is not directly involved with activation of an electrogenic Na^+/K^+ pump, which should have been immediately inhibited by ouabain. Possible alternative mechanism for the repolarization could include receptor desensitization or an increased K^+ conductance. However, the Na^+/K^+ pump in these cells clearly has an electrogenic component as shown by the small initial, ouabain induced depolarization (4 mV) and by the large (30 mV) transient hyperpolarization seen when ouabain was removed (Fig. 4*b*). This large transient hyperpolarization is presumably caused by Na^+ loading of the cells in the presence of ouabain, causing increased activation of the electrogenic component of the Na^+/K^+ pump after removing the ouabain¹⁶.

The L-Glu-induced depolarization of cells shown to contain GFAP, the block of this response by Na^+ removal, and the depolarization caused by L- or D-Asp and KA all show that astrocytes in primary culture are directly depolarized by excitatory amino acids via a Na^+ -dependent mechanism. This conclusion differs from that inferred from previous studies both *in vivo* and *in vitro*, where depolarization of glial cells was either not seen after L-Glu application^{3,4}, or, when it was observed, was ascribed to increased external K^+ released from stimulated neurones^{5,6}. However, the depolarization caused by L-Glu of non-excitatory cells in guinea pig cortical slices, also attributed to increased external concentration of K^+ , was followed by a distinct hyperpolarization¹⁷. This is similar to the response we have invariably observed in cultured astrocytes, suggesting that this effect may indeed occur in the mammalian CNS *in situ*. Also, L-glutamate-induced depolarization of non-mammalian glial cells from axon-free *Necturus* optic nerve¹⁸ and Schwann cells of axon-free squid giant nerve fibre sheaths¹⁹ has also been observed suggesting that this response is a highly conserved glial property. The present findings of amino acid-induced depolarization of astrocytes in culture can now be added to a growing list of neurotransmitter induced effects in glial cultures of which the majority have been identified by changes in cellular cyclic AMP content or by ligand binding studies^{20–24}. However, α -adrenergic receptor induced depolarization of glia in explant cultures²⁵ or of astrocytes in primary cultures²⁶ has also been observed, as well as a tetrodotoxin-sensitive, veratridine-induced Na^+ channel in primary astrocyte cultures¹¹. It thus appears that the membrane potential of astrocytes, and other glial

cells^{18,19}, can no longer be thought of as being responsive only to changes in external K^+ concentrations²⁷.

This work was supported by grants NS19492 from NINCDS and BNS 821873 from NSF to H.K.K. We thank Dr J. Huston for bovine GFAP, Dr G. Banker for neuronal hippocampal cultures and Dr P. Del Vecchio for use of his Nikon Labophot microscope and goat anti-mouse IgG. We thank Drs C. J. Frangakis (Sterling-Winthrop Research Institute, Rensselaer, New York), D. O. Carpenter, J. French-Mullen, D. Martin, N. T. Slater, J. Swann and R. Waniewski (New York State Division of Research Laboratories, Albany, New York) for helpful comments. We also thank Mrs M. V. Frangakis for growing the cell cultures and Mrs E. P. Graham for typing the manuscript.

Received 3 April; accepted 23 July 1984.

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Millisecond activation of transducin in the cyclic nucleotide cascade of vision

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Cyclic GMP has been implicated as a messenger molecule involved in visual transduction¹. Photoexcited rhodopsin (R^*) binds to a multisubunit membrane protein called transducin (T) and stimulates the exchange of a bound GDP molecule for GTP. This leads to the release of the α -subunit of T with bound GTP (T_α -GTP), which activates a cyclic GMP phosphodiesterase²⁻⁶. The question arises as to whether the hydrolysis of cyclic GMP that results from activation of the phosphodiesterase is sufficiently rapid to be involved in visual excitation, which occurs on a time scale of ~2 s in the single-photon limit⁷. Previous studies have suggested that the cyclic GMP phosphodiesterase is activated in less than 100 ms at moderate light levels^{3,8,9}. We report here light scattering studies of magnetically orientated frog rod outer segments which show that a molecule of R^* catalyses the activation of a molecule of T in about 1 ms. Thus, hundreds of molecules can be activated within the response time of vision in the single-photon limit, and the formation of T_α -GTP is fast enough for it to be a key step in visual transduction.

The near-infrared turbidity of rod outer segment (ROS) suspensions changes following excitation with a visible light pulse¹⁰⁻¹⁶. Kühn and co-workers⁸ delineated three types of scattering changes by using reconstituted disk membranes containing different amounts of transducin: (1) an R^* signal due to the bleaching of rhodopsin, (2) a binding signal due to the association of R^* with T-GDP and (3) a dissociation signal due to the release of T-GTP from R^* following GTP-GDP exchange. We have further analysed the physical basis and kinetics of the dissociation signal which is of interest because it arises from an amplified event in the cyclic nucleotide cascade. We magnetically orientated frog ROS fragments in a 6-kG field at 45° to an infrared beam (Fig. 1). We achieved a high degree of orientation because of the large diamagnetic anisotropy of the multiple transmembrane α -helices of rhodopsin¹⁷⁻¹⁸. The lattice spacing (29.5 nm) and intensities of the first four meridional orders of diffraction of these fragments are virtually the same as those of intact ROSs¹⁹⁻²⁰. The lamellar organization of disks and the

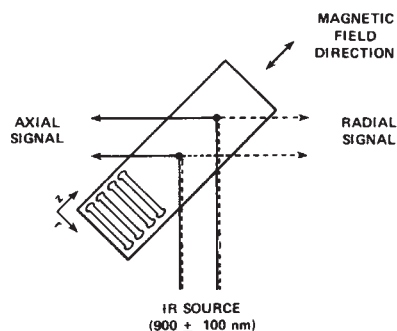


Fig. 1 Scattering geometry. ROS fragments in a 1-cm path length quartz cuvette were magnetically oriented at 45° to the incident 900 ± 100 nm IR beam (900 ± 100 nm). The intensity of light scattered at right angles to the incident beam was monitored by photodiodes located on the left and right sides by photovoltaic positive-intrinsic-negative diodes whose output was amplified, digitized and stored in 512 channels. The instrument response time was 0.1 ms. A photographic flash unit fitted with a 500-nm interference filter and a series of neutral density filters provided 0.5-ms light pulses to excite the sample via a light guide from below the cuvette. ROSs obtained from the retinas of freshly dissected dark-adapted frogs (*Rana pipiens* and *Rana catesbeiana*) were fragmented by being injected three times through a constricted 22-gauge hypodermic needle. This treatment made the plasma membrane sufficiently leaky to enable GTP to gain entry. These ROS fragments retained the native stacking of disks as judged by neutron scattering (measurement performed at ILL, Grenoble). A photodiode in the forward direction monitored the transmitted light intensity. The 500-nm excitation flash came from below via a light guide. Only axial changes (along the z direction) are seen by the detector on the left, whereas only radial changes (along the r direction) are seen by the detector on the right. The paths of the incident and scattered beams from scattering centres x and x' are shown.

orientation of these ROS fragments (Fig. 1) allowed us to obtain new information concerning the activation of transducin. The amplitude dA of the light scattered at right angles on the left and right sides by a volume element dV located at P is given by the product of the scattering power $f(x)$ dV and a phase factor:

$$dA(\text{left}) = f(x) \exp[-4\pi iz \sin(\pi/4)/\lambda] dV \quad (1)$$

$$dA(\text{right}) = f(x) \exp[-4\pi ir \sin(\pi/4)/\lambda] dV \quad (2)$$

where z and r are the axial and radial coordinates, respectively, and λ is the wavelength of incident light. Now let dV move in the axial (z) direction from position x to x' . It is evident from equations (1) and (2) that $dA(\text{left})$ will change because of the z -dependence of the exponential phase factor term, whereas

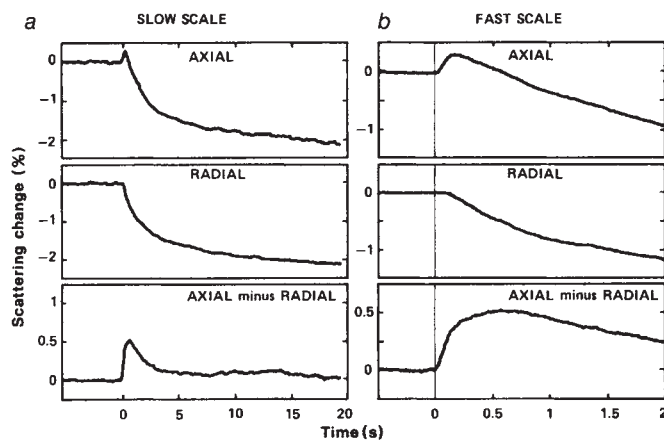


Fig. 2 Kinetics of the axial and radial light scattering changes and of their difference. A light pulse at $t=0$ produced $R^* = 3.4 \times 10^{-4}$ (mol fraction). The sample contained about 10^6 ROS fragments (obtained from one retina) in 2 ml Ringer's solution (102 mM NaCl, 2.7 mM KCl, 1.9 mM CaCl_2 , 2.1 mM MgSO_4 , 2 mM NaHCO_3 , 0.36 mM NaH_2PO_4 , pH 7.4), 0.5 mM GTP, 23 °C. *a*, Slow time scale; *b*, fast time scale. The axial signal has a fast and a slow component whereas the radial signal has only the slow component. The slow scattering decrease has the same magnitude (2%) and decay time (4 s) in both axial and radial signals. *a*, *b* Bottom panels, axial signal minus the radial signal showing the rapid axial component alone. This signal has a rise time (initial time constant of the rising phase) of 100 ms and a decay time (initial time constant of the falling phase) of 4 s.

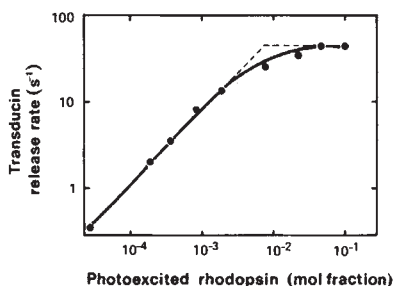


Fig. 3 Dependence of the transducin release rate on the mol fraction of photoexcited rhodopsin. GTP concentration, 0.5 mM. The release rates were determined from the slopes of the rise of the axial minus radial signals (for example, the bottom panel of Fig. 2*b*).

$dA(\text{right})$ will stay the same. Conversely, if dV is moved along the radial direction, $dA(\text{right})$ will change, whereas $dA(\text{left})$ will stay the same because the optical path length is unaltered. Thus, the detector on the left side monitors displacements in the axial direction only, whereas the one on the right side monitors displacements in the radial direction only. A displacement of molecules within the ROS fragment can be distinguished from a loss of molecules into the large aqueous space surrounding the fragments. In that event, $f(x)$ will be less and so $dA(\text{left})$ and $dA(\text{right})$ will decrease equally. A loss of scattering material from ROS fragments also leads to an increase in the intensity of transmitted light as monitored by a detector in the forward direction. A more detailed discussion of the characteristics of this θ - 2θ scattering experiment is given elsewhere²⁴.

The light scattering changes produced by a light pulse forming 3.4×10^{-4} R^* in the presence of 0.5 mM GTP are shown in Fig. 2. The axial signal is a combination of two components: a fast, transient scattering increase and a slow scattering decrease. The radial signal, in contrast, shows only a slow scattering decrease (Fig. 2). This slow decrease exhibits a lag of the same duration as the rise of the rapid axial signal.

The rapid axial increase in scattering probably arises from the release of transducin from the disk membrane surface into the inter-disk space following its activation to the GTP form.

The decrease in scattering seen in both the radial and axial signals is probably due to the loss of soluble transducin from the ROS fragments. This loss of scattering material reduces the contrast between the ROS fragment and the surrounding medium by decreasing the refractive index of the fragment and slightly increasing that of the medium. Hence, a general decrease in scattering is observed in all directions. We will refer to the rapid axial increase as the release signal and to the slow decrease as the loss signal. The loss signal is 20–30 times larger than the release signal because the 900-nm incident beam is more effective in probing changes occurring on the scale of hundreds of nanometres than of a few nanometres. Several lines of evidence support this interpretation. (1) The release signal and loss signal show the same dependence on nucleotide and R^* as does the activation of transducin. GTP or a hydrolysis-resistant analogue such as GTP γ S or GppNHP is required for the appearance of both signals. ATP, GDP and GMP are ineffective. The amount of R^* needed to elicit these signals is much less than the amount of transducin, indicating that R^* has a catalytic role in the generation of these signals. (2) A release of transducin into the inter-disk space would give the observed exclusively axial signal because such a movement changes the z but not the r coordinate of the scattering units. (3) A loss of transducin from the ROS fragments would decrease the axial and radial scattering equally, as observed, because such a movement removes scattering material and thereby lowers the scattering density. (4) Complementary information was obtained by monitoring the intensity of transmitted light with a detector in the forward direction. An increase in the relative intensity of transmitted light having the same time course and amplitude as the loss signal was observed. This increase is the complement of the decrease observed by the left and right detectors, confirming that this signal is due to the loss of scattering material (T-GTP) from the ROS fragments. (5) Supernatants of ROS fragment suspensions illuminated in the presence of GTP γ S contained all three subunits of transducin, as shown by SDS-polyacrylamide gel electrophoresis. In the absence of GTP γ S or illumination, transducin remained bound to the ROS fragments, as expected from previous studies²¹. (6) Small ROS fragments ($<10 \mu\text{m}$ in length) separated from large ones by centrifugation display a more rapid loss signal, as would be expected if their plasma membranes were rendered more leaky by extensive shearing. The kinetics of the release signal, in contrast, are independent of fragment size. (7) The loss signal exhibits a lag that matches the rise of the release signal. These signals are coupled because transducin must be released into the inter-disk space before it can leak out of the ROS fragment.

The loss signal corresponds to the dissociation signal observed by Kühn *et al.*⁸ in their light scattering study of isotropic suspensions of homogenized bovine ROSs. The kinetics of their dissociation signal (~ 100 ms) was more rapid than the kinetics of our loss signal because of the small size and absence of a barrier to diffusion in their photoreceptor fragments.

The rise time of the release signal depends on the concentration of photoexcited rhodopsin. The rate of release k , expressed in units of mol fraction transducin released per s, is given by $k = \beta/\alpha$, where β is the maximal slope of the rise of the release signal (measured from the axial minus radial signal and expressed in units of % scattering change per s), and α is its maximal amplitude (% scattering change) in saturating conditions (1% bleach and 0.5 mM GTP). The dependence of the transducin release rate on the concentration of R^* is shown in Fig. 3. The release rate is linearly proportional to R^* at low light levels, ranging from 0.35 s^{-1} at $R^* = 3.6 \times 10^{-5}$ to 13 s^{-1} at $R^* = 1.8 \times 10^{-3}$. The number of molecules of transducin activated by a molecule of R^* can be calculated from the slope of the linear region of the release rate versus R^* plot. A release rate of 1 s^{-1} at $R^* = 10^{-4}$ corresponds to 1,000 molecules of transducin activated per s per molecule of R^* , because the molar ratio of rhodopsin to transducin is 10. Thus, a molecule of transducin is activated to the GTP form by a molecule of R^* in ~ 1 ms. At high light levels, the release rate becomes independent

of R^* , reaching a maximal value of 45 s^{-1} , which corresponds to a rise time of 22 ms. This limit is imposed by a step after the formation of metarhodopsin II, the photolytic intermediate that serves as R^* (refs 22, 23), which occurs in 2 ms in our experimental conditions (23°C).

It is of interest to compare the rate of activation of transducin with the kinetics of visual excitation. In the single-photon limit, the closure of sodium channels and consequent hyperpolarization of the plasma membrane are relatively slow, occurring in times of 1–2 s⁷. Extrapolation of our measurements to the single-photon limit shows that a single molecule of R^* can activate 500 molecules of transducin in 0.5 s. Hence, the activation of transducin to the GTP form and its release into the inter-disk space occur sufficiently rapidly to enable T-GTP to serve as an amplified intermediate in visual excitation.

This work was supported by research grants from the National Eye Institute and the National Institute of General Medical Sciences (to L.S.) and the Comité des Membranes Biologiques, DGRST (to M.C.). T.M.V. was the recipient of an NIH training grant award (GM-07276).

Received 21 May; accepted 15 August 1984.

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Electrogenic Na–Ca exchange in retinal rod outer segment

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Previous work has suggested that a Na–Ca exchanger may have a key role in visual transduction in retinal rods^{1–10}. This exchanger is thought to maintain a low internal free Ca^{2+} concentration in darkness^{4–10} and to contribute to the rod's recovery after light by removing any internally released Ca^{2+} (refs 1, 2, 6–8). Little else is known about this transport mechanism in rods. We describe here an inward membrane current recorded from single isolated rods which appears to be associated with such external Na^+ -dependent Ca^{2+} efflux activity. External Na^+ , but not Li^+ , could generate this current; high external K^+ inhibited it while small amounts of La^{3+} ($10\text{ }\mu\text{M}$) completely abolished it. The exchanger can also transport Sr^{2+} , but not Ba^{2+} or other divalent cations. The exchange ratio was estimated to be $3\text{Na}^+:1\text{Ca}^{2+}$. As well as demonstrating clearly the Na–Ca exchanger in the rod outer segment, our experiments also cast serious doubt on the commonly held view that light simply releases internal Ca^{2+} to bind to and block the light-sensitive conductance.

We recorded the Na–Ca exchange current from an isolated, dark-adapted rod after its outer segment was loaded with Ca^{2+}

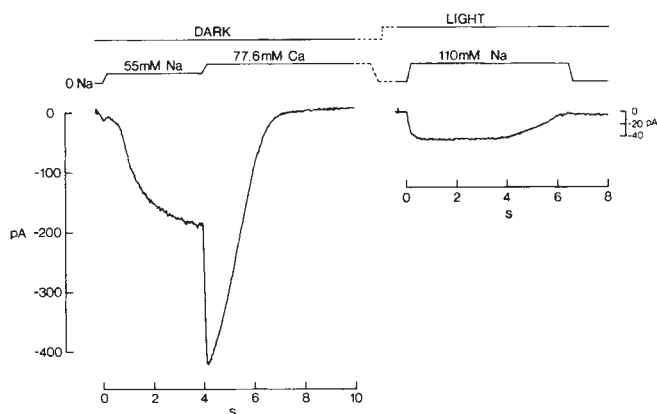


Fig. 1 Electrogenic Na^+ -dependent Ca^{2+} efflux, or Na–Ca exchange, recorded from a toad rod after Ca^{2+} loading. Under infrared light a piece of retina from a dark-adapted toad (*Bufo marinus*) was finely chopped in Ringer's solution, yielding some isolated rods with intact outer and inner segments⁷. Membrane current from one of these rods was recorded by sucking its inner segment into a close-fitting glass pipette containing Ringer's solution while its outer segment was rapidly perfused^{6,7,9}. Recordings were made with a current-to-voltage converter connected to the suction pipette. Time courses of solution changes, as indicated by junction currents⁷, were $\sim 200\text{ ms}$ and are shown above the sweeps. The valve controlling solution changes was operated pneumatically and with electronic timing so that the solution changes could be exactly reproduced many times. The exchange current was measured as the difference in recorded current on restoring Na^+ to a Na^+ -free solution, done in light before and after the Ca^{2+} load. The light-sensitive current in darkness was obtained as the difference between total current recorded in darkness (light-sensitive current plus junction current) and junction current recorded in a repeated run in light. Outward membrane current across the outer segment is plotted upwards on the ordinate scale. All solutions except the isotonic (77.6 mM) Ca^{2+} solution contained $\sim 1\text{ }\mu\text{M}$ buffered Ca^{2+} , with the following composition (in mM): 110 Na^+ or choline in partial or full substitution, 2.5 K^+ , 1.5 Mg^{2+} , 0.093 Ca^{2+} , 119.7 Cl^- , 2 Mg-EDTA , 5 TMA (tetramethylammonium)–HEPES, 5 dextrose , $\text{pH } 7.6$. The isotonic Ca^{2+} solution contained (in mM): 77.6 CaCl_2 , 5 TMA-HEPES , 5 dextrose , $\text{pH } 7.6$. The time lapse between Ca^{2+} loading and subsequent extrusion was $\sim 26\text{ s}$. Recordings were low pass-filtered at 100 Hz , 8-pole; room temperature. Steady light for junction current and exchange current measurements delivered about 800 photons (500 nm) $\mu\text{m}^{-2}\text{ s}^{-1}$, unpolarized. Dark Ca^{2+} loading was 588 pC , or $\sim 1.8 \times 10^9\text{ Ca}^{2+}$ ions.

(Fig. 1). Ca^{2+} loading was readily achieved as the light-sensitive conductance was highly permeable to divalent cations, especially after initial exposure to a Na solution containing low Ca^{2+} (refs 7, 9, 10). In order to isolate the exchange current, the recording was made normally in the presence of light which completely shut off the light-sensitive conductance; this conductance is thought to be the only ionic conductance in the plasma membrane of the outer segment^{11,12}. The current, which was inward and transient, was seen only after the outer segment was loaded with Ca^{2+} , and not with monovalent cations such as Na^+ , K^+ or Li^+ . However, loading with Sr^{2+} (but not Ba^{2+} or other divalent cations such as Mg^{2+} or Mn^{2+}) also induced a similar current. We observed detectable but substantially smaller inward currents when much smaller Ca^{2+} or Sr^{2+} loads were effected by omitting the initial exposure to low external Ca^{2+} before introducing isotonic Ca^{2+} or Sr^{2+} .

The size of the current depends on the transmembrane electrochemical gradients for both Na^+ and Ca^{2+} , and the total charge transfer due to the current appears to depend only on the Ca^{2+} load (Fig. 2). These observations suggest that the current is associated with electrogenic Na^+ -dependent Ca^{2+} efflux rather than ion movement through a novel membrane conductance being turned on by a rise in internal Ca^{2+} (ref. 13). The inward direction of the current suggests that more than two Na^+ ions enter the outer segment in exchange for each Ca^{2+} ion extruded. Assuming that the Ca^{2+} load is entirely removed

Fig. 2 Dependence of Na-Ca exchange current on size of Ca^{2+} load. The Ca^{2+} load was controlled by light which completely shut off the dark Ca^{2+} influx at various times, as shown by traces *a-d*. All records are from the same cell. All solutions and experimental conditions were the same as in Fig. 1, the only other difference being that the Na^+ -free solution was not interposed between the 77.6 mM Ca^{2+} and the 110 mM Na^+ solutions in order to minimize the time lapse between Ca^{2+} load and subsequent extrusion. The broken line indicates the time course of decline of the Na^+ current if 0 Na^+ 110 mM Choline is introduced instead of 77.6 mM Ca^{2+} . The Ca^{2+} loads (Q_{load}) were *a*, 562; *b*, 404; *c*, 230; and *d*, 139 pC, equivalent to $\sim 1.7 \times 10^9$, 1.2×10^9 , 6.9×10^8 and 4.2×10^8 Ca^{2+} ions respectively. The charge ratios $Q_{\text{load}}/Q_{\text{exchange}}$ were *a*, 2.38; *b*, 2.02; *c*, 1.87; and *d*, 2.43 (mean 2.18). This ratio is related to the stoichiometry (n) of the exchange (that is, $n \text{ Na}^+ : 1 \text{ Ca}^{2+}$) by $Q_{\text{load}}/Q_{\text{exchange}} = 2/(n-2)$. Hence n was 2.92. From 18 experiments the average value of n was 2.94 ± 0.10 ($\pm$ s.d.). (In Figs 1 and 3, n was not computed because of the relatively long time between Ca^{2+} loading and readmission of external Na^+ (26-111 s).) Other experiments have indicated that a long time lapse tends to allow some of the Ca^{2+} influx to slowly leave the outer segment via an alternative route, most probably by diffusion into the inner segment (see also ref. 8).) Steady light delivered 300 photons (500 nm) $\mu\text{m}^{-2} \text{ s}^{-1}$, unpolarized; this produced $\sim 4.5 \times 10^3$ photoisomerizations s^{-1} , assuming an effective collecting area of $15 \mu\text{m}^2$ (see ref. 22). Light itself should also trigger some Ca^{2+} efflux even in the absence of Ca^{2+} loading^{1,2,8}. From estimates obtained by others^{1,2,8}, this additional efflux even at high light intensities is unlikely to be over 10^7 Ca^{2+} ions s^{-1} , corresponding to an inward exchange current of ~ 1.6 pA. However, most of this current should already have been subtracted from the records because each record was obtained as the difference between two runs in the same light conditions (Fig. 1 legend). As the exchange current induced by a Ca^{2+} load as reported here is typically 30-40 pA, the error introduced to the above analysis by the light-triggered Ca^{2+} efflux should be less than a few per cent.

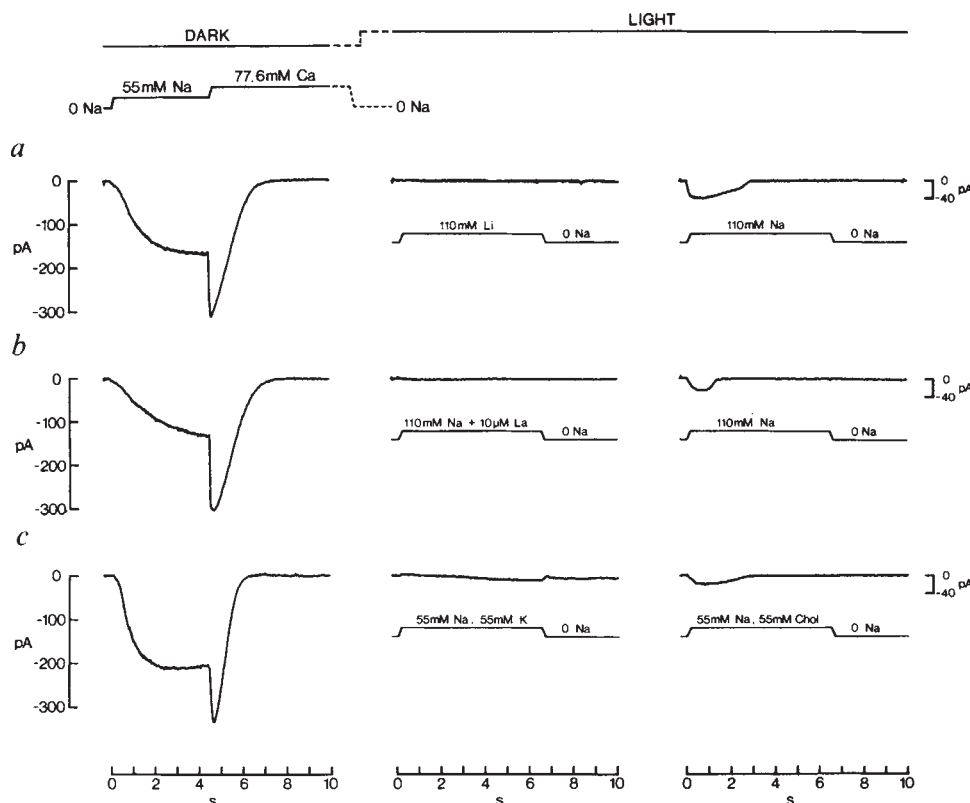
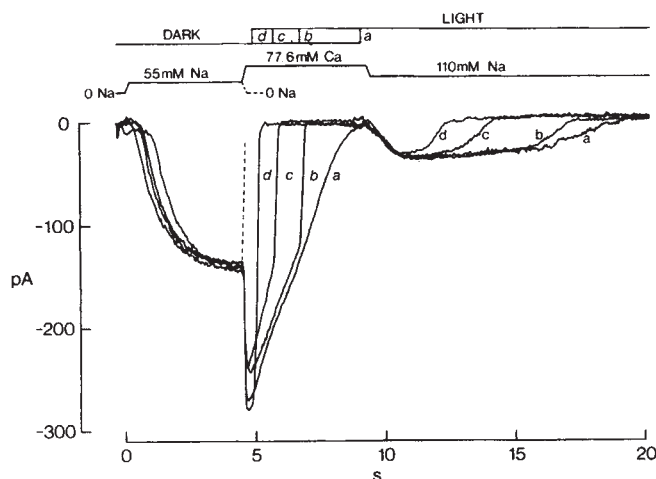


Fig. 3 Effects of external Li^+ , La^{3+} and K^+ on the Na-dependent Ca^{2+} extrusion. Three different cells in *a*, *b* and *c*. In each experiment, the test solution containing Li^+ , La^{3+} or high K^+ was followed by a control Na^+ solution. *a*, *c*, Na^+ (including 0 Na^+) and Li^+ solutions identical in composition to those in Fig. 1 with a buffered Ca^{2+} concentration of $\sim 1 \mu\text{M}$. *b*, Na^+ solutions contained nominally (that is, unbuffered) zero Ca^{2+} as follows (in mM): 110 Na^+ , 2.5 K^+ , 1.6 Mg^{2+} , 0 Ca^{2+} , 115.7 Cl^- , 5 TMA-HEPES, 5 dextrose, pH 7.6. Time lapse between Ca^{2+} load and admission of test solution: *a*, 37 s; *b*, 57 s; *c*, 33 s. Time lapse between Ca^{2+} load and admission of control Na^+ solution: *a*, 73 s; *b*, 111 s; *c*, 51 s. Steady light delivered 800 Photons (500 nm) $\mu\text{m}^{-2} \text{ sec}^{-1}$, unpolarized.

by the exchanger on restoration of external Na^+ , the coupling ratio is estimated to be close to $3\text{Na}^+ : 1\text{Ca}^{2+}$ (Fig. 2 legend). Since the Ca^{2+} loading and the subsequent extrusion by the exchanger could be repeated reversibly many times on the same cell, the above assumption seems reasonable. It has previously been suggested² that an uncoupled Ca^{2+} efflux might also exist at the outer segment; such a mechanism should generate an outward membrane current, however, which we have not detected. If present, this uncoupled Ca^{2+} efflux probably saturates at very low capacity and produces an outward current too small to be detected in our experimental conditions. The exchange ratio of $3 \text{ Na}^+ : 1 \text{ Ca}^{2+}$ is similar to some estimates obtained from other types of cells^{14,15}.

We have examined several properties of this exchange current (Fig. 3) and found that external Li^+ cannot support the Ca^{2+}

efflux in place of Na^+ , high external K^+ inhibits the efflux, and trace amounts of La^{3+} completely abolish it. These results confirm indirect evidence from earlier experiments about the actions of these ions on rods^{4-7,9,10,16} and they suggest also that this exchanger in rods may share some similar properties with that found elsewhere, for example squid axon^{17,18}.

One striking feature of the Na-Ca exchanger in rods is its large Ca^{2+} pumping capacity. In the experiment of Fig. 1, for example, calculations show that the Ca^{2+} influx during exposure to isotonic Ca^{2+} should have increased the total Ca^{2+} concentration within the outer segment by $\sim 2 \text{ mM}$; yet it took the exchanger only seconds to remove this excess Ca^{2+} and allow recovery of the cell. We observed rather similar effectiveness in pumping when a normal (1 mM) instead of low Ca^{2+} concentration was present externally during Ca^{2+} extrusion. In normal

circumstances, when the steady Ca^{2+} influx through the light-sensitive conductance in darkness is likely to be relatively small^{9,10}, the exchanger should run well below its maximal capacity.

Collective evidence so far^{4,5,7,9,10,19-21} has clearly indicated that a rise in internal Ca^{2+} somehow leads to the blockage of the light-sensitive conductance. In view of this, it is rather surprising that the large Ca^{2+} current in, for example, Fig. 1 switches off relatively slowly despite a peak influx of as many as 10^9 Ca^{2+} ions s^{-1} ; thus, the current declines only by ~50% after $1.4 \times 10^9 \text{ Ca}^{2+}$ ions have entered. It might be argued that Ca^{2+} buffering within the rod outer segment is so extremely rapid and powerful as to be able to maintain low internal free Ca^{2+} despite this very large influx. This, however, seems highly unlikely because we found that much smaller Ca^{2+} loads in the same conditions also led to a large increase in exchange current in darkness (data not shown), suggesting a rapid rise in free Ca^{2+} within the outer segment. Internal Ca^{2+} most probably

does not act by binding directly to and blocking the conductance, or perhaps it requires additional cellular substrates to express fully its blocking action. In contrast, the large Ca^{2+} current could be completely shut off within ~300 ms by light at an intensity causing about 5×10^3 photoisomerizations s^{-1} in the rod outer segment (Fig. 2). From other estimates^{1,2,8}, any rise in internal free Ca^{2+} triggered by the light during this period is probably only ~ 10^7 ions, far less than that caused by the Ca^{2+} influx through the light-sensitive conductance. This suggests that the ability of light to block the conductance results from its modulation of other cellular substrates which act more directly or perhaps more effectively on the conductance.

We thank Professors Alan Hodgkin, Denis Baylor, L. E. Moore and Drs Peter McNaughton and Brian Nunn for discussions. We also thank Dr Kenneth Nielsen for help in computer programming. This work was supported by the US National Eye Institute and the Retina Research Foundation, Houston, Texas.

Received 12 June; accepted 14 August 1984.

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Abrogation of resistance to bone marrow transplantation by induction of specific tolerance in natural killer cells?

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Hybrid resistance describes the capacity of first generation (F_1) hybrids between certain mouse strains to inhibit the growth of tumour or haematopoietic cells of parental origin^{1,2}. The cells that appear to mediate this phenomenon differ from classical T and B lymphocytes in several respects. For example, they are unusually radioresistant³, show no immunological memory⁴, are present in thymectomized⁵ or congenitally athymic⁶ mice, and are not functional until about 3 weeks after birth^{3,4}. These characteristics suggest that the effectors are natural killer (NK) cells⁷. Although most of the evidence implicating NK cells in hybrid resistance is circumstantial, the experiments of Warner and Dennert⁸ are more direct in that they show that resistance can be restored to mice with a congenital or induced defect in NK activity by the infusion of cells belonging to an NK clone. Conversely, treatment of mice with an antibody to NK cells abrogated hybrid resistance to parental bone marrow grafts⁹. Both NK cells and the effectors of hybrid resistance are generally considered to be nonspecific. We have now investigated this assumption by attempting to prevent hybrid resistance by neonatal tolerance induction with parental strain antigens. Our data indicate that hybrid resistance can be abrogated by this means and that the tolerance is specific and transferable with Thy-1^+ spleen cells.

Groups of $(\text{CBA/Ca} \times \text{C57BL/Gr})F_1$ hybrid mice ($\text{H-2}^{k/b}$) received varying doses of adult C57BL/Gr bone marrow cells intraperitoneally within 24 h of birth and were tested 2-3 months later for their capacity to resist the engraftment of parental or F_1 hybrid bone marrow cells following lethal whole body irradiation (8.0 Gy). The 'take' of the grafts was assessed by the incorporation of ^{125}I -labelled 5-iodo-2'-deoxyuridine in the recipient spleens (see Fig. 1 legend for experimental details).

Hybrid resistance is clearly demonstrated in Fig. 1. Normal F_1 recipients of C57BL bone marrow failed to incorporate significant levels of the label (Fig. 1c) compared with mice that had received syngeneic F_1 cells (Fig. 1a). The neonatal inoculation of C57BL cells had no effect on the engraftment of F_1 cells (Fig. 1b) but prevented rejection of C57BL cells (Fig. 1d). Although in this experiment the take of C57BL bone marrow cells was virtually the same as that of F_1 cells, it tended to be lower in most other experiments (see Fig. 2). Neonatal exposure to parental strain bone marrow antigens thus had a profound effect on the development of hybrid resistance. Unexpectedly, cells from the SJL/J strain (H-2^s), which are likewise rejected by $(\text{CBA} \times \text{C57BL})F_1$ mice (Fig. 1e), were unaffected by the tolerance (Fig. 1f). This result, which has been obtained consistently in eight separate experiments, shows conclusively that the tolerance mechanism operative in this system is strain specific and suggests that haematopoietic resistance is mediated by more than one population of effector cells.

The dose of cells inoculated neonatally varied between 1.0 and 4.0×10^7 . The data in Fig. 1 were based on 1.5×10^7 cells, but in the other seven experiments 4.0×10^7 cells were used; we have found that 1×10^7 cells are inadequate (data not shown). Female recipients were generally more resistant to tolerance induction: 1.5×10^7 induced tolerance in only some females but 4×10^7 secured tolerance in all.

Three attempts to break tolerance by the adoptive transfer of 10^8 normal syngeneic F_1 hybrid spleen cells were unsuccessful, suggesting that tolerance is maintained by active suppression. We therefore injected 10^8 spleen cells from tolerized mice into normal syngeneic F_1 animals to ascertain whether tolerance in hybrid resistance is transferable. The cell recipients were irradiated 6 days after cell transfer and received the indicator bone marrow grafts on the following day (see Fig. 2 legend). As found in previous experiments, hybrid resistance to C57BL grafts was abrogated by neonatal tolerance induction (Fig. 2, compare a, b and c). The adoptive transfer of cells from tolerant donors conferred unresponsiveness to C57BL (Fig. 2d) but not to SJL (Fig. 2e) cells. Pretreatment of the cells with a monoclonal anti- Thy-1.2 antibody and complement completely prevented the transfer of tolerance (Fig. 2f), showing that Thy-1^+ cells, presumably T lymphocytes, are required. Similar results have been obtained in three other experiments.

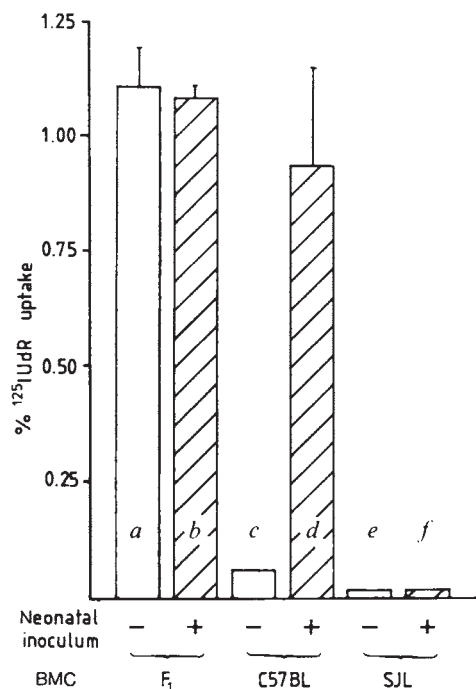


Fig. 1 Abrogation of hybrid resistance in (CBA × C57BL)F₁ hybrid mice by the neonatal inoculation of C57BL bone marrow cells (BMC). 8–12-week old hybrid mice that had received 1.5×10^7 C57BL bone marrow cells intraperitoneally (i.p.) within 24 h of birth (groups *b*, *d*, *f*) were used to measure hybrid resistance. Groups of four male mice were exposed to 8.0 Gy whole body X-irradiation and given intravenously (i.v.) 5×10^5 bone marrow cells from F₁ hybrid (*b*), C57BL (*d*) or SJL (*f*) donors (tolerized animals shown as hatched bars in this and other figures). Controls were provided by non-tolerized and sex and age-matched F₁ hybrids (*a*, *c*, *e*) given the same types of cells, respectively. Engraftment of donor cells was assessed by measuring the incorporation of ¹²⁵I-labelled 5-iodo-2'-deoxyuridine (¹²⁵IUdR) in the recipient spleens¹⁷ as follows. Five days after bone marrow cell transplantation, the mice were given a single i.p. dose of 25 μg 5-fluorodeoxyuridine to decrease the availability of endogenous thymidine. This was followed 1 h later by 0.5 μCi ¹²⁵IUdR i.p. Spleens were removed 18–24 h later and counted in a Packard γ spectrometer. ¹²⁵IUdR uptake was calculated as the % of total radioisotope injected. Geometric means and standard deviations of the arithmetic means are shown for each group. In some experiments (though not in the experiment shown here), engraftment of parental CBA cells was also evaluated: such data were always comparable to the syngeneic controls.

Our study raises the question of the antigens involved in hybrid resistance. Genetic analysis by Cudkowicz and Nakamura¹⁰ has shown that in certain mouse strains the expression of hybrid resistance is controlled by a number of *Hh* (haematopoietic histocompatibility) loci, some of which are linked to *H-2*, the major histocompatibility complex (MHC) of the mouse. *Hh* products seem to be expressed only in homozygous inbred strains. Both C57BL and SJL strains express products of the *Hh-1* locus which are thought to be highly cross-reactive, if not identical¹¹. The specificity we have encountered is therefore all the more intriguing. One important difference between the antigenic relationship of the two strains to their hybrid hosts is that SJL cells are completely histoincompatible, whereas C57BL cells are not. It could therefore be argued that mechanisms other than those mediating NK activity are involved in hybrid resistance to allogeneic haematopoietic grafts, although it is generally accepted that the effectors responsible for resistance to allogeneic haematopoietic grafts in lethally irradiated animals are similar, if not identical, to those causing the rejection of parental cells⁷.

In this connection, we have shown in two experiments (Fig. 3) that (CBA × SJL)F₁ bone marrow cells are not rejected by normal (CBA × C57BL)F₁ mice, indicating that conventional

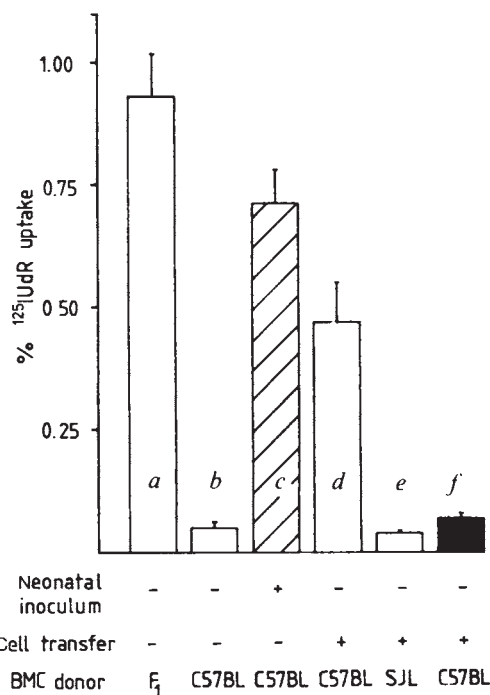
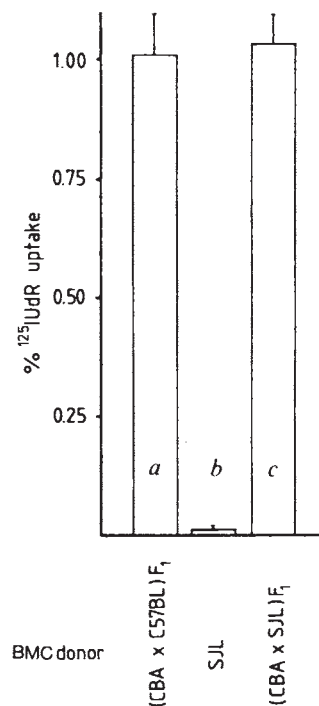


Fig. 2 Suppression of hybrid resistance by the adoptive transfer of spleen cells from tolerant mice. Tolerant F₁ hybrid donors were prepared as described in Fig. 1 legend except that the tolerizing inoculum consisted of 4×10^7 C57BL bone marrow cells. 10^8 spleen cells from tolerized donors were injected i.v. into normal adult (CBA × C57BL)F₁ mice 6 days before lethal whole body irradiation (groups *d*–*f*), followed 1 day later by 5×10^5 C57BL bone marrow cells given i.v. Group *f* received spleen cells that had been pre-treated by two exposures to a monoclonal IgM anti-Thy-1 antibody and guinea pig complement¹⁶. Control mice were either normal (*a*, *b*) or neonatally tolerized (*c*) F₁ hybrids. Resistance to engraftment by C57BL (*b*–*d*, *f*) or SJL (*e*) cells was assessed by ¹²⁵IUdR uptake (see Fig. 1 legend) and compared with controls given syngeneic bone marrow cells (*a*). All groups comprised four mice.

Fig. 3 Resistance of (CBA × C57BL)F₁ hybrid mice to haematopoietic grafts presenting SJL strain MHC antigens. Engraftment of bone marrow cells was determined in groups of four 8–12-week old (CBA × C57BL)F₁ hybrids following lethal irradiation and reconstitution with 5×10^5 bone marrow cells from syngeneic (group *a*), SJL (*b*) or (CBA × SJL)F₁ (*c*) mice as described in Fig. 1 legend.



SJL MHC antigens are unlikely to be the target of resistance. Another approach was to examine resistance to parental grafts in (C57BL $\times$ SJL) F_1 hybrids. In an early experiment we found that such hybrids accepted C57BL bone marrow cells following tolerance induction with C57BL cells but retained resistance to bone marrow from SJL mice (the other parental strain). However, other experiments on these lines showed such marked variability in the take of both parental and even of syngeneic F_1 hybrid cells that we cannot have confidence in this result.

Our experiments show that T lymphocytes can suppress hybrid resistance, but we have not excluded the possibility that resistance to parental and allogeneic bone marrow cells is mediated by the same nonspecific NK effector and that specificity is imparted by suppressor lymphocytes. Were this the case, it would be expected that SJL cells would take when transplanted in the same inoculum with C57BL cells, to (CBA $\times$ C57BL) F_1 hybrids that had been tolerized with C57BL antigens. Figure 4 illustrates that this is not the case. Here we have used two doses of bone marrow cells (2.5×10^5 and 2.5×10^6) to increase the sensitivity of the assay. SJL cells at the higher dose were mixed with C57BL cells at the lower dose and inoculated into tolerized (CBA $\times$ C57BL) F_1 recipients. The take of this mixture (Fig. 4h) was not significantly different from the take of 2.5×10^5 C57BL cells alone (Fig. 4g). If exposure to C57BL antigens had caused the activation of a suppressor cell specific for C57BL, which then acted on a nonspecific effector, the take of cells in group h should have been greater than that in group d (2.5×10^6 C57BL cells in normal recipients) and approaching the level of incorporation observed in group b (2.5×10^6 F_1 cells). These experiments therefore favour the notion that the effectors for SJL and C57BL are distinct in their specificity.

We believe this to be the first demonstration that hybrid resistance can be prevented by neonatal tolerance induction and that specific Thy-1 $^+$ suppressor cells can apparently regulate the activity of NK cells *in vivo*. The specificity of the effector cells is compatible with the earlier report by Cudkowicz and Bennett³ that adult (C3H $\times$ C57BL) F_1 hybrids (H-2 $^{k/b}$) become specifically unresponsive to H-2 b bone marrow cells after multiple immunization with C57BL spleen cells. It is at variance with the recent observation (ref. 11 and I. Nakamura, personal communication) that homozygous H-2 s and H-2 b lymphoid cells were equally effective in abrogating resistance to C57BL bone marrow cells when given to (C57BL/6 $\times$ DBA/2) F_1 adult mice shortly before irradiation and reconstitution. However, this apparent lack of specificity could be due to the use of a hybrid (H-2 $^{b/d}$) different from that used by Cudkowicz and Bennett and by us (H-2 $^{k/b}$): whereas the latter recognizes at least two epitopes of Hh antigens, one common to H-2 s and H-2 b strains and the other unique to H-2 s , the former may recognize only the common epitope. Unresponsiveness to the common determinant in (C57BL/6 $\times$ DBA/2) F_1 mice could therefore be expected to allow the take of H-2 s as well as of parental grafts. Alternatively, the suppression rather than the effector mechanism might have been nonspecific in the system described by Nakamura.

It could be argued that the specificity of the NK cells is imparted by naturally occurring antibody, based on the model of Chow *et al.*¹² for NK surveillance against syngeneic tumours. Such an interpretation is virtually ruled out by the observation that hybrid resistance is intact in mice depleted of B lymphocytes by the neonatal administration of anti- μ antibody¹³.

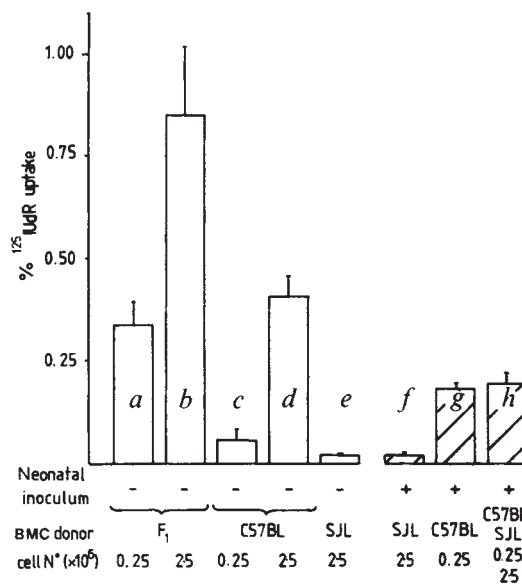


Fig. 4 Resistance of (CBA $\times$ C57BL) F_1 hybrid mice, tolerized neonatally to parental C57BL Hh antigens, to allogeneic SJL bone marrow cells injected as a mixture with C57BL bone marrow cells. (CBA $\times$ C57BL) F_1 mice tolerant to C57BL bone marrow cells were prepared as described in Fig. 2 legend (groups f-h). Groups of four 8-10-week old (CBA $\times$ C57BL) F_1 hybrids were lethally irradiated (8.0 Gy) and given i.v., 24 h later, bone marrow cells from F_1 (a, b), C57BL (c, d, g) or SJL (e, f) donors. Groups a, c and g were given 2.5×10^5 and groups b, d and e 2.5×10^6 cells. Group h received a mixture of 2.5×10^5 C57BL and 2.5×10^6 SJL cells. Engraftment was assessed 5 days after reconstitution as described in Fig. 1. Although we were unable to include SJL $\rightarrow$ SJL controls in this experiment to show that the SJL bone marrow cells take normally in susceptible hosts, we have found in five other experiments that this is indeed the case.

In conclusion, our evidence shows that resistance to parental and allogeneic haematopoietic grafts is mediated by different effector cell populations, presumed to be subpopulations of NK cells. Moreover, the effectors involved in hybrid resistance can be controlled by specific suppressor T cells. Our demonstration of specificity in hybrid resistance probably accounts for the unexplained finding by Warner and Dennert⁸ that cloned NK cells from C57BL/6 mice did not establish resistance against C57BL/6 bone marrow grafts in NK-deficient (C57BL/6 $\times$ C3H) F_1 hybrids (H-2 $^{b/k}$), despite the fact that resistance to allogeneic BALB/c grafts (H-2 d) was restored.

Our model should enable us to examine the effect of tolerance induction on the activity of NK cells *in vitro*, as well as on the progression of syngeneic tumours, some of which seem to be subject to NK cell surveillance^{8,14}. Finally our results make it highly probable that the transplantation of allogeneic bone marrow grafts *in utero*^{15,16} should not be thwarted by fetal NK activity.

We thank the Wellcome Trust for their support of L.S.R. and the MRC for M.W.'s postgraduate studentship. We also thank Ms Beverley Whittle, Mrs Ellen Yanez and Mr M. Gevaux for their assistance and Professor W.L. Ford for helpful comments.

Received 4 June; accepted 23 August 1984.

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Because of the possible presence of introns in the gene, we isolated the corresponding cDNA to elucidate the complete structure of the LHRH precursor protein.

A 600-base pair (bp) DNA restriction fragment obtained from the genomic clone and containing the LHRH coding sequence (J.P.A. *et al.*, in preparation) was used as a probe to screen a large cDNA library constructed in λ phage²³. As human hypothalami were not available to us, the cDNA was derived from human placental tissue where LHRH synthesis has been shown to occur²⁴. Two hybridizing clones were isolated which contained cDNA inserts of ~1,500 and 800 bp in length. Figure 4 shows the complete nucleotide sequences of both cDNAs, together with the deduced amino acid sequence of the LHRH precursor protein.

The most prominent feature of the cDNA is a very long 5' untranslated region of >1,000 nucleotides. A similarly sized 5' region has been described for the human preproenkephalin B gene²⁵ but seems otherwise to be a rare phenomenon. As LHRH is expressed in various tissues, transcription of the precursor gene from different tissue-specific promoters²⁶ might be invoked to account for the presence of such an extended 5' region in the placental cDNA^{26,27}. The trivial explanation, that the clone is derived from an unspliced nuclear RNA, can be refuted because two introns present in the gene had been clearly removed (J.P.A. *et al.*, in preparation). The smaller of the two cloned cDNAs has ~400 nucleotides of 5' untranslated sequence which also contain the best consensus splice acceptor site²⁸ (nucleotides 960-974), making the possibility of a partially spliced version unlikely. The very low abundance of the corresponding mRNA in placental tissue has so far precluded the use of Northern analysis²⁹ to establish the correct length of its 5' region. Furthermore, complete co-linearity of this region with the established genomic sequence (J.P.A. *et al.*, in preparation) excludes artefacts which might have been introduced during reverse transcription and molecular cloning.

The largest open reading frame (nucleotides 1,063-1,350) specifies the coding sequence for LHRH and translates into a peptide of molecular weight ~10,000 with characteristics of a precursor for a polyfunctional protein (for review see ref. 30). This reading frame is terminated by an ochre stop codon and followed by 160 nucleotides of a 3' untranslated region containing an AATAAA sequence for polyadenylation²⁷ shortly upstream of the poly(A) tail. The actual start of translation within the open reading frame is unclear. The first ATG initiation codon (position 1,063) is followed by an in-frame ATG four codons downstream. This second ATG, but not the first, is followed by a purine and preceded by an A at position -3, as is the case with most eukaryotic initiation codons³¹. We therefore consider this latter ATG at position 1,075 to be the most likely start codon for initiation of the synthesis of the LHRH precursor protein although it involves a violation of the rule which specifies that the first ATG should bear this function³². It is possible that both ATGs are involved in the initiation of protein synthesis, resulting in a low translational efficiency³¹.

The first 23 amino acids form a typical signal sequence containing a hydrophobic middle section³³. The putative signal sequence ends in two serine residues followed by the expected decapeptide sequence for LHRH^{1,2}. Cleavage of the signal peptide results in an amino-terminal glutamic residue which spontaneously or enzymatically undergoes cyclization to pyro-Glu, the modified amino-terminus of LHRH^{1,2}. The decapeptide sequence is followed by a glycine, the standard donor of the amino group for C-terminal amidation¹⁷. A Lys-Arg cleavage site is present for enzymatic processing, as is found in many precursors to neuroendocrine peptides³⁰. Thus, the isolated cDNAs code for a true precursor protein to LHRH which features a direct linkage of signal sequence to biologically active peptide reminiscent of the situation in the precursor for vasopressin³⁴.

The remainder of the LHRH precursor consists of a peptide of 56 amino acids, the function of which is unknown. It does not contain any sites for *N*-glycosylation (Asn-X-Thr/Ser) and

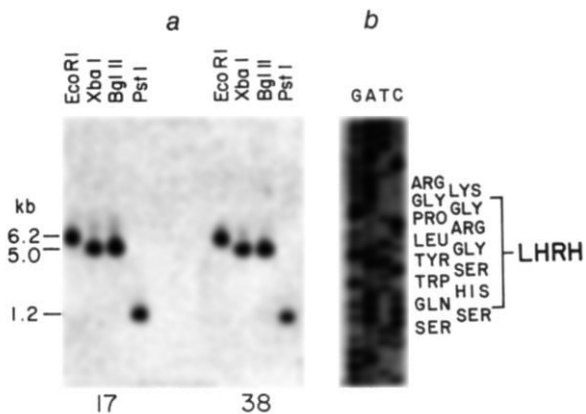


Fig. 3 Identification of a cloned human genomic DNA segment carrying LHRH coding sequences. Sequential screening of a human genomic library²² with two pools of oligomers (long (38) and short (17) probes; see Fig. 1) yielded one isolate the DNA of which hybridized with both oligomeric probes. **a**, Southern analysis⁴⁰ of the DNA digested with four restriction endonucleases (*EcoRI*, *XbaI*, *BglII*, *PstI*). DNA fragment sizes in kilobase pairs (kb) are indicated on the left and were assessed using *HindIII*-cleaved λ DNA (BRL). **b**, A sequencing gel (G, A, T, C) across the region of hybridization with both oligomer pools. The amino acid sequence encoded by the nucleotide sequence in this region is indicated.

Methods: 10^6 λ phage containing human genomic DNA were screened on duplicate filters using the long probes (see Fig. 1) which had been phosphorylated to a specific activity of 10^7 c.p.m. per pmol. Hybridization was at 37 °C in the presence of 30% formamide using 10^7 c.p.m. per filter. Filters were washed in $3 \times \text{SSC}$ ($1 \times \text{SSC} = 0.15 \text{ M NaCl}$, $0.015 \text{ M Na-citrate}$) at 37 °C and exposed to X-ray film. Phage from 300 small areas corresponding to the number and location of positive signals were replated on individual plates and probed with long and short probes (Fig. 1). Short probe hybridization was at room temperature in the presence of 20% formamide and corresponding filters were washed at the same temperature in $5 \times \text{SSC}$. DNA from the single isolate which hybridized with both pools of oligomers was cleaved with restriction enzymes and DNA fragments characterized by Southern⁴⁰ analysis using the two oligonucleotide pools as probes under the conditions specified above. A 1,200-bp DNA fragment containing the hybridizing region was cloned into phage M13 mp18 RF-DNA and recombinant single-stranded DNA was sequenced using primed DNA synthesis in the presence of dideoxynucleoside triphosphates^{21,47}.

no homologies were found when the sequence was compared with other known hypothalamically produced precursors to neuroendocrine peptides³⁴⁻³⁷. Interestingly, the C-terminus of this peptide consists of the sequence Lys-Lys-Ile, which may represent an enzymatic cleavage site for further processing. It is likely that the resulting peptide of 53 amino acids is released together with LHRH, possibly to complement the biological function of the decapeptide in ways not yet understood. It might be a carrier protein of LHRH in analogy with neurophysins^{34,38} or it could be involved in the release of follicle-stimulating protein (FSH) from pituitary gonadotrophs, a process for which an agent other than LHRH has been invoked³⁹.

The LHRH precursor produced in placenta and hypothalamus is probably the same, as antisera raised against a synthetic peptide derived from its sequence can be used to stain rat hypothalamic LHRH-producing neurones (H. Phillips *et al.*, in preparation). In addition, Southern analysis⁴⁰ of the human genome using the cloned cDNA as a probe did not reveal the presence of related genes (J.P.A. *et al.*, in preparation). The two other hypothalamic releasing factors for which cloned cDNAs exist, namely growth hormone releasing factor and corticotropin releasing factor, are also encoded by single genes^{36,37}. However, the molecular weight of 10,000 (10K) for prepro-LHRH as deduced from the cloned cDNA is much smaller than the 28K precursor reported from *in vitro* translation and immunoprecipitation of rat hypothalamic mRNA⁴¹. There are two possible

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Developmentally regulated production of platelet-derived growth factor-like molecules

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Platelet-derived growth factor (PDGF) is thought to mediate the proliferation of smooth muscle cells in injured arteries, and may be involved in the pathogenesis of atherosclerosis¹. PDGF-like molecules from non-platelet sources may also play a role in the regulation of cell activity in other circumstances. Transformation of cells by a wide range of oncogenic agents appears to activate a cellular gene encoding a PDGF-like molecule²⁻⁶, possibly accounting for the ability of transformed cells to grow without addition of exogenous mitogens⁷⁻¹². We show here that a molecule (PDGF-c) which can compete with ¹²⁵I-PDGF for binding to PDGF receptors is secreted by cultured rat aortic smooth muscle cells (rASM) isolated from 13 to 18-day-old rats (pups) but not from three-month-old animals (adults). Thus, production of PDGF-c appears to be developmentally regulated and may be a factor in the more rapid proliferation of rASM and synthesis of connective tissue components which occurs during growth of the aorta *in vivo*.

Pup rASM secrete into their culture medium an average of 170 times (Range 77-1,100×) more PDGF-c than do adult rASM (Table 1). This difference is consistently observed for two separate isolates (pup and adult) of rASM from each of three different rat strains. Prior adsorption of pup rASM-conditioned medium with goat anti-PDGF IgG covalently coupled to Sepharose-4B completely abolishes the ability of pup rASM-conditioned medium to reduce ¹²⁵I-PDGF binding to human fibroblasts (data not shown), demonstrating that pup rASM PDGF-c is antigenically related to PDGF. Pup, but not adult, rASM continue to produce PDGF-c during 20 passages in culture over a period of at least six months. These data suggest that the critical events occurring *in vivo* between the ages of two weeks and three months is not a mere consequence of the passage of time or number of cell divisions. Pup rASM-conditioned

medium is mitogenic for 3T3 fibroblastoid cells (Fig. 1), whereas adult rASM-conditioned medium is nonmitogenic or only weakly so (data not shown). Stimulation of DNA synthesis by added PDGF or pup rASM-conditioned medium is, however, completely prevented by simultaneous addition of goat anti-PDGF IgG (Fig. 1). Comparable amounts of non-immune goat IgG have no effect. We conclude from these results that pup rASM PDGF-c is mitogenic as well as antigenically similar to PDGF.

Isolates of pup rASM possess considerably fewer binding sites for ¹²⁵I-PDGF compared with adult rASM; the binding which does occur increases linearly over the ¹²⁵I-PDGF concentration range tested (Fig. 2). This binding may be to a previously unrecognized PDGF-binding site with a dissociation constant substantially greater than that of the PDGF mitogen receptor. At concentrations of ¹²⁵I-PDGF near to the apparent dissociation constant of the PDGF mitogen receptor (6.6×10^{-12} M), adult rASM isolates bind 11-26 times more ¹²⁵I-PDGF than pup rASM from the same strain (Fig. 2). We also measured ¹²⁵I-PDGF binding to human foreskin fibroblasts (6.5×10^4 receptors/cell) and to A431 carcinoma cells (no detectable receptors/cell), for comparison with rASM (Fig. 2a).

PDGF-receptor complexes are rapidly internalized at 37 °C, resulting in a marked reduction in membrane receptors available for binding subsequently added ligand¹³⁻¹⁷, a process referred to as down regulation^{18,19}. Since pup rASM secrete PDGF-c, any PDGF receptors produced by these cells may be continuously down regulated by endogenously produced PDGF-c. To examine this possibility, we determined the effect of PDGF and rat pup PDGF-c on the binding properties of test cells (Table 2). Human fibroblasts preincubated at 37 °C with PDGF or pup rASM-conditioned medium (which contains PDGF-c) retain very few binding sites for ¹²⁵I-PDGF. The level of ¹²⁵I-PDGF binding to adult rASM preincubated with PDGF or pup rASM-conditioned medium was reduced to levels approaching that of pup rASM (Table 2). Medium conditioned

Table 1 Rate of PDGF-c production by rASM

Rat strain	Rate of PDGF-c production (ng per equiv.)	
	Pup	Adult
Sprague-Dawley		
Isolate 1	1.13	0.036
Isolate 2	1.09	0.01
Wistar Kyoto		
Isolate 1	1.92	0.002
Isolate 2	2.25	0.004
Spontaneous hypertensive rat		
Isolate 1	3.48	0.003
Isolate 2	0.48	0.006
Average $\pm$ s.d.	1.73 $\pm$ 1.07	0.01 $\pm$ 0.013

Aortic smooth muscle cells were placed into culture as described elsewhere³⁰. The PDGF-c concentration in media conditioned (Ref. 6 for method) by two different isolates of rASM obtained from 13- to 18-day-old or 3-month-old rats of three strains (Sprague-Dawley, Wistar Kyoto, and the spontaneously hypertensive rat) was determined by radioreceptor assay^{18,37} using volumes of conditioned medium resulting in an approximately 50% decrease in specifically bound ¹²⁵I-PDGF (radiolabelled as previously described^{13,37}). The results were normalized by expressing production rate as ng PDGF-c per equivalent (where an equivalent equals 1 ml medium conditioned by 1×10^6 cells for 48 h). The tabulated values are the averages of at least two, and in most cases three or more, determinations of PDGF-c concentration.

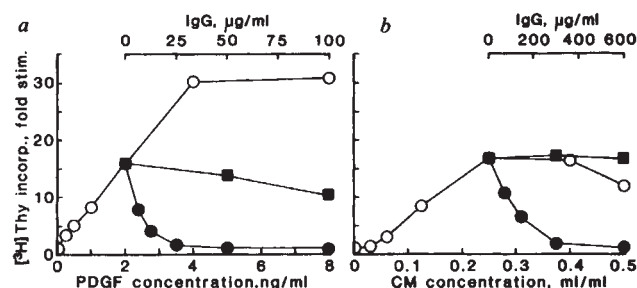


Fig. 1 Stimulation of ³H-thymidine incorporation by pup rASM conditioned medium. Swiss 3T3 D-1 cells were plated onto Costar 24-well cluster trays in DMEM containing 10% calf serum, grown to confluence and then incubated for two days in DMEM containing 2% bovine plasma-derived serum. The medium was replaced with 1 ml fresh DMEM supplemented with 2% bovine plasma-derived serum containing the following additions. a, Open circles, increasing concentrations of PDGF; closed circles, 2 ng PDGF and increasing concentrations of goat anti-PDGF IgG; closed squares, 2 ng PDGF and increasing concentrations of goat non-immune IgG. b, Open circles, increasing concentrations of pup rASM-conditioned medium; closed circles, 0.25 ml ml⁻¹ CM and increasing concentrations of goat anti-PDGF IgG closed squares, 0.25 ml ml⁻¹ CM and increasing concentrations of goat non-immune IgG [Monospecific antiserum to pure PDGF was prepared in a goat. (Elaine Raines, personal communication). IgG was prepared by sodium sulphate precipitation and DEAE chromatography]. The cells were incubated with test substance for 19 h, 37 °C and then ³H-thymidine incorporation determined as described previously¹³. Results are expressed as the ratio of ³H-thymidine incorporation by cells exposed to test mixtures to incorporation by untreated cells. The plotted values are the means of triplicate determinations from a representative experiment. The pup rASM-conditioned medium was produced by cells isolated from 16-day-old Sprague-Dawley rats and contained 3.06 ± 0.54 (s.d.) ng ml⁻¹ PDGF-c. CM, conditioned medium.

Table 2 PDGF receptor expression by rASMC and human fibroblasts after exposure to pup rASMC-conditioned medium or PDGF

Cell type	Test substance (amt per ml)	Specific ^{125}I -PDGF binding (c.p.m. per 10^6 cells)
a Human fibroblasts	0	34,300
	2 ng PDGF	0
	1 ml Pup CM	2,400
	1 ml Adult CM	20,500
b Pup rASMC	0	900
c Adult rASMC	0	5,400
	2 ng PDGF	1,400
	1 ml Pup CM	2,600
	1 ml Adult CM	5,900

Pup rASMC-conditioned medium was obtained from cells isolated from 18-day-old Sprague-Dawley rats and contained 1.12 ng ml^{-1} of PDGF-c. Adult rASMC-conditioned medium was obtained from cells isolated from 3-month-old spontaneously hypertensive rats and contained 0.026 ng ml^{-1} PDGF-c. Human adult foreskin fibroblasts were prepared as for the radioreceptor assay^{18,37} and rASMC were plated onto Costar 24-well cluster trays in DMEM containing 20% fetal bovine serum, grown to confluence, then incubated in DMEM containing 2% bovine plasma-derived serum. Two days later the cells were exposed to 1 ml binding medium, 1 ml binding medium containing 2 ng ml^{-1} PDGF, or 1 ml rASMC-conditioned medium supplemented with 0.025 M HEPES, pH 7.4 for 4 h, 37°C . The cells were washed three times with binding rinse and then exposed for 2 h, 4°C to 1 ml binding medium containing 0.5 ng ml^{-1} ^{125}I -PDGF. Specifically bound radioactivity was determined as described in Fig. 2 legend, and cell counts were obtained from parallel cells treated with binding medium alone. Numbers are the mean of triplicate wells from a representative experiment. Since pup rASMC do not express detectable high affinity receptors for ^{125}I -PDGF (see Fig. 2) they were only examined for their ability to bind ^{125}I -PDGF following exposure of the cells to binding rinse alone. CM, conditioned medium.

by adult rASMC (which does not contain PDGF-c) had a minimal effect on ^{125}I -PDGF binding to human fibroblasts and no effect on its binding to adult rASMC. Therefore, PDGF-c production by pup rASMC may account for our inability to detect high affinity PDGF receptors on pup rASMC.

We find that adult and pup rASMC express approximately 1.0×10^4 ^{125}I -EGF binding sites per cell with an estimated K_D of $6.7 \times 10^{-10} \text{ M}$ (data not shown). There was no detectable difference in the number of EGF receptors expressed by pup and adult rASMC, unlike the PDGF receptor. Pup rASMC-conditioned medium contains no detectable (less than 0.4 ng/ml) EGF-like binding competitor activity as determined by radioreceptor assay (data not shown). The ability of anti-PDGF IgG to completely abolish the stimulation of DNA synthesis by 3T3 cells caused by pup rASMC-conditioned medium (Fig. 1), also indicates that these cells do not secrete biologically significant amounts of non-PDGF-like mitogens for 3T3 cells. Therefore, it appears that the mitogen-related phenotypic differences between adult and pup rASMC are relatively specific for PDGF and do not reflect general differences in mitogen receptor number or in production of growth factors.

A similar phenomenon may occur in human fibroblasts since Clemmons²³ has reported that, in platelet-poor plasma, normal human fetal fibroblasts secrete a factor which enhances their own growth. This does not occur in fibroblasts from three-year-old donors. Pup rASMC, but not adult rASMC, secrete a PDGF-like mitogen and express fewer detectable PDGF receptors. This is consistent with our suggestion that rASMC synthesize the PDGF mitogen receptor at all developmental stages and only pup rASMC synthesize a PDGF-c which provides a constant mitogenic stimulation, resulting in down regulation of their own PDGF receptors. That pup rASMC can respond to the PDGF-c they produce is indicated by the ability of PDGF to stimulate

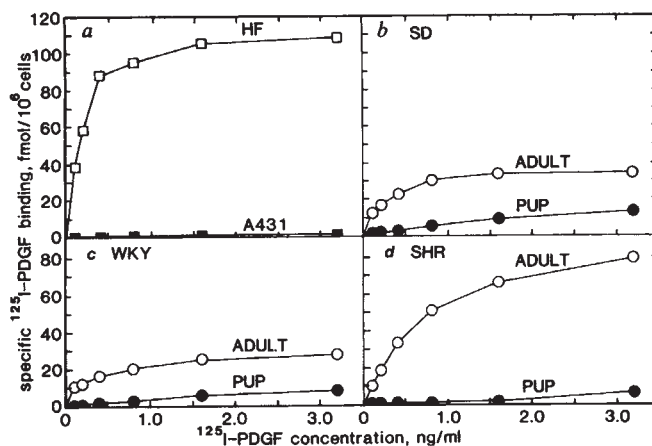


Fig. 2 Specific binding of ^{125}I -PDGF to rASMC. Cells were plated onto 24-well trays in Waymouth's medium containing 10% or 20% bovine serum or in DMEM containing 10% or 20% fetal bovine serum. After cells had grown to near confluence the media were replaced with DMEM supplemented with 2% PDGF-deficient calf serum (CMS-I; see ref. 38) or 2% bovine plasma-derived serum, and assayed for ^{125}I -PDGF binding 2 to 3 days later as previously described^{13,37}. Cell counts were obtained from cells treated in a parallel fashion but with binding medium alone and nonspecifically bound c.p.m., determined by exposing cells to ^{125}I -PDGF and an excess of unlabeled, partially-purified PDGF ($5\text{--}40 \mu\text{g ml}^{-1}$, the equivalent of 50 to 400 ng ml^{-1} purified PDGF), was subtracted to determine the amount of specifically bound ^{125}I -PDGF. The plotted values represent the average amount of ^{125}I -PDGF binding to three or more different isolates of rASMC from each age (except for SHR pup, $n=2$). HF, human fibroblasts; A431, human carcinoma cells; SD, Sprague-Dawley; WKy, Wistar Kyoto; SHR, spontaneously hypertensive rat.

[^3H]thymidine incorporation in some, but not all, isolates of pup rASMC (data not shown). Nilsson *et al.*²⁰ have also reported that rASMC obtained from 5-day-old donors and cultured in serum-free medium respond to added PDGF with increased DNA synthesis and more rapid growth. Thus we suggest that pup rASMC may regulate their growth properties by producing a growth factor (PDGF-c) to which they themselves respond—that is, an autocrine mechanism first proposed to explain the growth properties of transformed cells^{21,22}.

The basic structure of the rat aorta is already developed at birth^{24,25}. From birth until three months of age, however, there is a substantial increase in cell number^{26–30} and the amount of connective tissue matrix components^{24–27}. PDGF-c could be responsible for these changes since PDGF has been shown to stimulate the growth of cultured smooth muscle cells^{13,31–33} and enhance the production of connective tissue components *in vitro*^{34,35}. PDGF-c may thus provide an example of autocrine stimulation of growth operating during normal development and which can be inappropriately activated during neoplastic transformation.

We thank Linda Mohai and Karen Tittle for technical assistance and Anna Choi and Jan Fullenwider for typing the manuscript. This research was supported by NIH grants HL 18645 and HL 29873 and a grant from R. J. Reynolds Industries, Inc.

Received 18 May; accepted 6 August 1984.

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A novel transforming gene in a human malignant melanoma cell line

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Cellular transforming genes can be detected in human tumours by DNA-mediated transfection into NIH 3T3 mouse fibroblasts^{1,2}. The activated transforming genes have been, in most cases, members of the *ras* gene family, of which the most frequently found is the c-Ki-ras oncogene³⁻⁷ and least frequently the c-Ha-ras gene⁸⁻¹⁰. An increasing number of studies has identified the presence of activated N-ras (which has no known viral homologue) in human tumour cell lines¹¹⁻¹⁸. Furthermore, other transforming genes, distinct from the *ras* gene family, have been reported in B- and T-cell lymphomas^{19,20}. The activation of c-Ha-ras and N-ras has been described in some cell lines derived from cases of human malignant melanoma²¹. Here we describe the presence of transforming activity in the DNA from a human melanoma cell line which shows weak homology with members of the *ras* oncogene family.

High-molecular weight DNA prepared from established melanoma cell lines was used to transform NIH 3T3 cells by the calcium phosphate co-precipitation method²². Table 1 shows the results of these transfection assays. The foci observed were collected and grown to confluence, then DNA was extracted and analysed by Southern blotting²³ for human repetitive sequences using Blur 8 (ref. 24), a clone of human *Alu* sequences (Fig. 1), and total human DNA (data not shown). Positive results were obtained for foci derived from lines NKI4, MeWo and Mel Swift. (The Mel Swift line was subsequently found to

contain an activated N-ras gene and will be reported separately.) DNA from NKI4 and MeWo transformants was further analysed by Southern blotting and hybridization to clones containing v-Ha-ras (BS9)²⁵, v-Ki-ras (HiHi-3)²⁶ and N-ras (pAT 8.8)¹⁴. In addition, these DNA samples were blotted and hybridized to probes specific for the following oncogenes: B-lym, v-abl, v-sis, v-erb, v-fes, v-fgr, v-fms, v-fps, v-fos, v-mos, v-myb, v-myc, v-raf, v-src and v-yes. In each case the hybridization pattern obtained was identical to that of normal NIH 3T3 DNA. DNA from serially transformed foci (second cycle transfectants) from NKI4 showed a conserved 16-kilobase (kb) *Eco*RI fragment containing *Alu* sequences (Fig. 1).

Primary and secondary transformants from NKI4 were grown in soft agar (0.3% Noble) and colonies were detected after 2 weeks. Plating efficiencies well in excess of 10% were observed whereas control NIH 3T3 cells had negligible colony-forming efficiencies. Colonies were picked from the soft agar cultures and grown to confluence in liquid medium. DNA was extracted and Southern analysis demonstrated that the *Alu* sequences associated with NIH 3T3 transformation were conserved in cells displaying the anchorage-independent phenotype.

DNA was isolated from a secondary transformant of the NKI4 cell line and enriched for the 16-kb *Eco*RI fragment by size fractionation. This fragment was cloned into λ L47 and one clone was characterized in detail (λ NK/16). Hybridization of λ NK/16 to clones containing v-Ha-ras (BS9), c-Ha-ras¹⁰ (pT24-C3), Ki-ras (HiHi-3) and N-ras (pAT 8.8) under low stringency showed that λ NK/16 has some homology with HiHi-3 and c-Ha-ras (Fig. 2). Weak hybridization was observed when BS9 was probed with λ NK/16 (Fig. 2, lane b), but there was clearer evidence of conserved sequences between λ NK/16 and BS9 when the λ NK/16 clone was probed with BS9 (Fig. 3, lanes d, e). There was no apparent hybridization of λ NK/16 probe to the first exon of N-ras (Fig. 2, lane d) and λ NK/16 failed to hybridize with a probe specific for the first exon of N-ras (data not shown). As the first exon of the known *ras* genes is highly

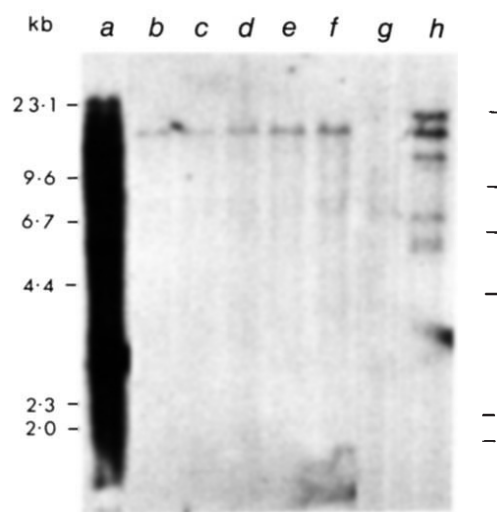


Fig. 1 Detection of human repetitive sequences. 20 μ g of high-molecular weight DNA isolated from primary and secondary transformants were digested with *Eco*RI, electrophoresed in horizontal 0.8% agarose gels and blotted onto nitrocellulose filters²³. The filters were hybridized in stringent conditions²⁹ to 10^6 c.p.m. ml^{-1} ³²P-labelled 0.3-kbp cloned repetitive sequence DNA purified from plasmid Blur 8 (ref. 24; specific activity $2-4 \times 10^8$ c.p.m. μg^{-1}). Filters were washed with $0.6 \times \text{SSC}$, 0.1% SDS at 60°C for 1 h. NKI4 transformants: lane a, primary focus; lanes b-f, secondary foci; lane g, NIH 3T3; lane h, EJ secondary focus. *Hind*III-digested λ c1857 DNAs served as molecular weight markers.

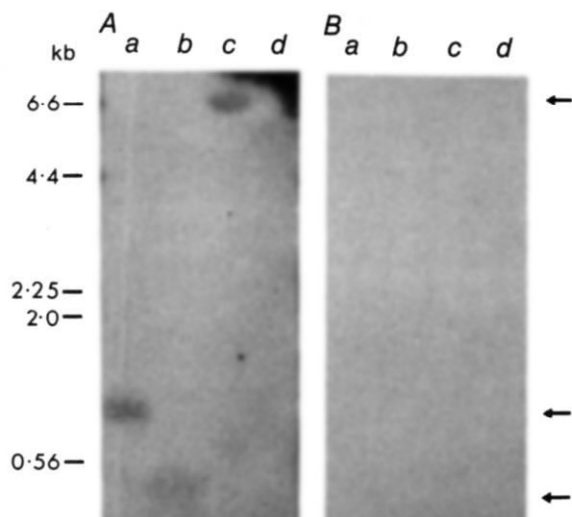


Fig. 2 Hybridization of λ NK/16 to members of the *ras* gene family. Lane *a*, v-Ki-*ras* (Hi-Hi 3)²⁶; *b*, v-Ha-*ras* (BS9)²⁵; *c*, c-Ha-*ras* (pT24-C3)¹⁰; *d*, N-*ras* (*Pvu*II fragment containing exon 1). Arrows indicate positions of cloned insert DNAs. *Hind*III-digested λ c1857 DNAs were used as molecular weight markers. 10 μ g (per lane) of cloned DNA were analysed by Southern blotting and hybridization to the purified insert of λ NK/16 clone under: *A*, low stringency conditions of 30% formamide, 6 $\times$ SSC, 1 $\times$ Denhardt's at 42 $^{\circ}$ C and washed in 2 $\times$ SSC, 0.1% SDS at 60 $^{\circ}$ C for 1 h, or *B*, high stringency conditions²³ and washed in 0.1 $\times$ SSC, 0.1% SDS at 60 $^{\circ}$ C.

conserved, we conclude that λ NK/16 must contain divergent sequences which show similarity to the HiHi-3, BS9 and pT24-C3 clones. Hybridization to these clones was lost in high stringency conditions (Fig. 2*B*).

The λ NK/16 clone was also hybridized in high stringency conditions to Southern filters containing the plasmid clones of those viral oncogenes mentioned above and to clones of the following cellular and viral oncogenes: v-*ski*, v-*ets*, v-*rel*, c-*sis*, c-*myc*, c-*myb* and c-*mos*. In each case, the plasmid clone had been digested with the restriction endonuclease(s) which separated the oncogene-specific fragment(s) from the remainder of the clone under electrophoresis. No hybridization was observed

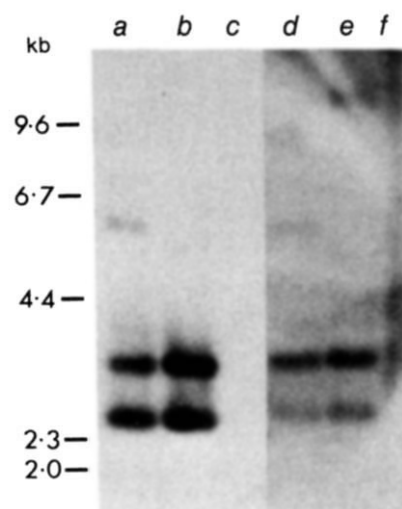


Fig. 3 Detection of human repetitive and *ras*-related sequences in λ NK/16. 1 μ g of cloned DNA was digested with *Kpn*I and *Pvu*II, electrophoresed on 0.8% agarose gels and blotted onto nitrocellulose filters. Lanes *a*-*c* were hybridized to Blur 8 in conditions of high stringency as described in Fig. 1 legend. Lanes *d*-*f* were hybridized to BS9 (v-Ha-*ras*) in conditions of low stringency as described in Fig. 2 legend. Lanes *a*, *d*, λ NK/16 insert; *b*, *e*, λ NK/16 clone; *c*, *f*, λ L47 *Hind*III-digested λ c1857 DNAs served as molecular weight markers.

with the coding sequences of these clones. In conditions of low stringency, faint hybridization was seen with the 1.9-kb pSA17 v-*abl* clone, but this was probably due to the presence of mouse sequences in the clone²⁷. The λ NK/16 clone detects fragments of 5.5 and 6.5 kb in *Eco*RI-digested human DNA and of 5.0 and 7.0 kb in *Eco*RI-digested dog DNA in high stringency conditions. This suggests that λ NK/16 contains conserved sequences. Furthermore, λ NK/16 also detects a human transcript of 3.5 kb on Northern blot analysis.

λ NK/16 probably contains only part of the gene as it does not cross-hybridize with all of the *ras* gene clones tested. Exon 1 of N-*ras*, for example, is not detected at all. Furthermore,

Table 1 Transforming activity of DNA from melanoma tumours and cell lines

	Transformation frequency, foci per μ g DNA (no. foci/no. flasks)		
	1st cycle	2nd cycle	3rd cycle
Controls			
3T3	0.03 (127/195)	ND	ND
Human peripheral blood leukocytes	0.05 (54/52)	ND	ND
Human placenta	0.02 (12/25)	ND	ND
Salmon sperm	0.03 (2/10)	ND	ND
EJ (bladder carcinoma)	0.13 (95/37)	>1.2 (>704/29)	>1.6 (>1,275/40)
Melanoma cell lines			
Mel 2a	0.05 (26/24)	ND	ND
Mel 28	0.01 (4/14)	ND	ND
Mel 57	0.03 (17/33)	ND	ND
Mel 64	0.05 (9/9)	ND	ND
RPM 1 5966	0.03 (17/29)	ND	ND
Mel Swift	0.04 (25/36)	0.16 (62/19)	0.16 (16/5)
MeWo	0.03 (26/33)	0.13 (76/30)	0.13 (13/5)
NK14	0.04 (29/33)	0.12 (49/20)	0.12 (63/26)

NIH 3T3 cells were seeded at 3×10^5 per 25 cm² flask and 24 h later exposed to 20 μ g DNA as a calcium phosphate precipitate²². Foci were counted 3 weeks after transfection. ND, not determined (when the 1st cycle transfectants contained no detectable human DNA, no further transfections were performed).

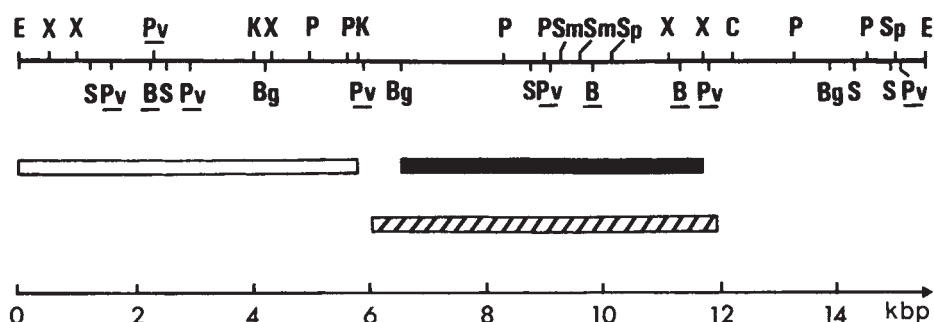


Fig. 4 Restriction map of λ NK/16. Restriction endonuclease sites are as follows: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; Sm, *Sma*I; Sp, *Sph*I; S, *Sst*I; X, *Xba*I. Underlined enzymes are incompletely mapped. *Hind*III and *Sal*I did not cut within the clone. Solid bar indicates regions hybridizing to human DNA. Open bar indicates the region containing mouse-related sequences. Hatched bar indicates region of homology with the v-Ha-*ras* (BS9) probe.

λ NK/16 is not active in the NIH 3T3 transfection assay—this lack of biological activity of λ NK/16 indicates that the entire activated gene has not been cloned. Comparison of the restriction map of λ NK/16 (Fig. 4) with those of the five known human *ras* genes (c-Ha-*ras*1, c-Ha-*ras*2, c-Ki-*ras*1, c-Ki-*ras*2 and N-*ras*^{15,28}) indicates that the sequences showing homology with *ras* are likely to be novel. Thus, we have demonstrated a weak homology of λ NK/16 with the *ras* gene clones (and not with any other cloned oncogenes), but proof that this gene is a member of the *ras* family must await sequence analysis.

We thank David Hughes, Althea Smallwood, Frederick Rayment, Julia Roberts and Jonathan Fletcher for technical assistance, and Dr Margaret Knowles for help with the biological assays. The cell lines Mel 2a, Mel 57, RPMI 5966, Mel Swift, MeWo and NK14 were provided by Dr J. Embleton. Our use of NK14 was by courtesy of Dr Jan de Vries. NIH 3T3 cells were provided by Demetrios Spandidos. Cloned oncogene probes were provided by Drs S. Aaronson, D. Baltimore, M. Bishop, G. Cooper, T. Curran, R. Gallo, A. Hall, J. Neil, D. Lowy, M. Nunn, M. Oskarsson, U. Rapp, C. Sherr, E. Stavnezer, N. Teich and M. Yoshida.

Received 16 July; accepted 30 August 1984.

An artefact explains the apparent association of the transferrin receptor with a *ras* gene product

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There is substantial evidence implicating *ras* genes in a number of human neoplasms¹. The *ras* genes of several human tumours²⁻⁶ display mutational changes which are likely to be responsible for their transforming activity. Normal cells also express *ras* genes⁷, over-expression of which can induce cellular transformation⁸. *ras* genes encode proteins of ~21,000 molecular weight (MW) (p21)⁹ that are localized to the inner surface of the plasma membrane^{10,11}. Much effort is being focused on the elucidation of the physiological function of *ras*-encoded proteins in normal and transformed cells, concentrating on interactions between p21 and other cellular elements. Recently, Finkel and Cooper¹² reported that p21 in extracts of human bladder carcinoma cells is involved in a molecular complex with the transferrin receptor of these cells. This report aroused considerable interest, particularly as expression of the transferrin receptor has been linked to cell proliferation¹³⁻¹⁵. I present here evidence that the apparent association of p21 and the transferrin receptor is an artefact of the immunoprecipitation technique¹².

Transferrin is an iron-binding protein present in serum at concentrations of 2–4 mg ml⁻¹ (ref. 16). The human transferrin receptor can be solubilized in buffers containing non-ionic detergents with the retention of transferrin-binding activity^{15,17,18} and the receptor has been purified by affinity chromatography on Sepharose to which transferrin was covalently linked¹⁹. When immune serum is used for coating the staphylococcal protein A-Sepharose (Pharmacia) to be used in immunoprecipitation, I observe that serum proteins other than IgG can become associated nonspecifically with the resin. Notably, some transferrin remains with the protein A-Sepharose even after extensive washing with buffers containing Triton X-100. When such coated and washed resin is boiled in sample buffer for SDS-polyacrylamide gel electrophoresis, the released proteins revealed by silver staining of the gel included albumin and transferrin in addition to the predominant heavy and light chains of IgG (data not shown). Polypeptides having the molecular weights of albumin and transferrin are not observed when uncoated protein A-Sepharose is similarly boiled and analysed. Thus, protein

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A-Sepharose coated with immune serum is in effect an affinity column for the transferrin receptor.

The observations that led Finkel and Cooper¹² to conclude that the transferrin receptor is associated with the *ras* gene product are consistent with inadvertent affinity purification of the transferrin receptor during p21 immunoprecipitation. To test this hypothesis, protein A-Sepharose coated in various ways was added in the absence of additional antibodies to a lysate of ³⁵S-methionine-labelled T24 bladder carcinoma cells. No band corresponding to the 90,000-MW transferrin receptor subunit was found to be associated with uncoated protein A-Sepharose (Fig. 1, lane b). This lysate does contain labelled transferrin receptor, demonstrated by inclusion of a monoclonal antibody (B3/25, Boehringer Mannheim) which recognizes the transferrin receptor (Fig. 1, lane a). B3/25 is bound by protein A and thus no coating of the resin is needed for immunoprecipitation of the transferrin receptor. Precoating of the protein A-Sepharose with an IgG fraction of rabbit anti-rat IgG (Cappel) results in an increase in antibody-independent association of several radioactive polypeptides from the cell lysate; most notably a band appears having the molecular weight of the transferrin receptor subunit (Fig. 1, lane c). This is most likely to occur as a result of transferrin present in the IgG fraction used to coat this resin. Indeed, when whole rabbit serum against rat IgG (Cappel) is used for coating the protein A-Sepharose, a significant increase is seen in the amount of receptor bound by the resin (Fig. 1, lane d), reflecting the higher transferrin content of the coating substance. The inclusion of rat anti-*ras* monoclonal, Y13-238 (a gift of M. Furth) results in the immunospecific binding of radiolabelled p21 from the lysate (Fig. 1, lane e), but no increase in the amount of transferrin receptor associated with the resin. Finkel and Cooper¹² used normal rat serum as a negative control for the rat anti-*ras* monoclonal antibodies. Therefore, the effect of normal rat serum was examined in the present study. When 20 µl of normal rat serum are used in place of the anti-*ras* antibody, not only is p21 no longer present but the transferrin receptor also disappears (Fig. 1, lane f). This latter effect is probably the result of transferrin in the normal rat serum. This rat serum transferrin seems able to compete with the rabbit transferrin on the coated resin, preventing the affinity binding of the transferrin receptor. In support of this contention, human diferric transferrin (100 µg ml⁻¹) also eliminates the receptor band when added to serum-coated protein A-Sepharose in the absence of additional antibodies (Fig. 1, lane h). It has long been recognized that extensive species cross-reactivity exists in the interaction of transferrin and its receptor^{20,21}. The addition of anti-*ras* monoclonal together with 20 µl of normal rat serum yields the expected result; p21 is immunoprecipitated by the anti-*ras*, but the transferrin receptor was prevented from interacting with the serum-coated protein A-Sepharose by the transferrin in the normal rat serum (Fig. 1, lane g). To demonstrate conclusively that the T24 lysate contained active transferrin receptor capable of being affinity purified, a portion of the labelled lysate is exposed to Sepharose covalently linked with human diferric transferrin (a gift of G. Ashwell). After washing and boiling the transferrin-Sepharose in analogy to the immunoprecipitations, the major polypeptide seen on the SDS gel is the 90,000-MW transferrin receptor subunit (Fig. 1, lane i). Addition of transferrin to the lysate prevents this intentional affinity chromatography of the receptor on transferrin-Sepharose (Fig. 1, lane j).

All our observations are consistent with the absence of any direct association between p21 and the transferrin receptor. When anti-*ras* is added in the absence of normal rat serum, p21 is precipitated with no increase in the amount of resin-associated transferrin receptor (compare Fig. 1, lanes d and e). Likewise, when anti-*ras* is added in the presence of normal rat serum, p21 is precipitated but there is no *ras*-specific precipitation of the transferrin receptor (compare lanes f and g). Unfortunately, Finkel and Cooper¹² used conditions analogous to lane f as a negative control for an anti-*ras* immunoprecipitation analogous

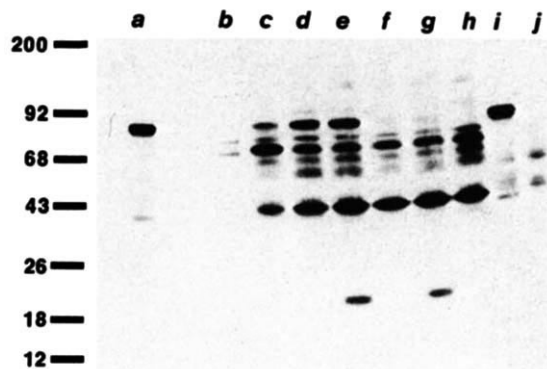


Fig. 1 Electrophoretic analysis of samples from the lysate of ³⁵S-methionine-labelled T24 cells. Lane a, material bound by an anti-transferrin receptor monoclonal antibody using uncoated protein A-Sepharose; lane b, material bound by uncoated protein A-Sepharose alone; lane c, material bound protein A-Sepharose coated with an IgG fraction of rabbit anti-rat immunoglobulin serum; lane d, material bound by protein A-Sepharose coated with rabbit anti-rat immunoglobulin serum; lane e, anti-*ras* monoclonal antibody added to lane d conditions; lane f, normal rat serum added to lane d conditions; lane g, anti-*ras* monoclonal antibody plus normal rat serum added to lane d conditions; lane h, transferrin added to lane d conditions; lane i, material bound by transferrin-Sepharose; lane j, transferrin added to lane i conditions. Numbers to the left of the gel represent molecular weights $\times 10^{-3}$ of marker polypeptides.

Methods: Human bladder carcinoma cells (T24) at 75% confluence were labelled in 100-mm culture dishes for 18 h by incubation with 400 µCi ml⁻¹ ³⁵S-methionine (Amersham) in 2.5 ml RPMI 1640 medium containing 10% fetal bovine serum and 10% of the normal amount of methionine. The labelled cells were washed twice with 10 ml ice-cold phosphate-buffered saline and then scraped into cell lysis buffer (2 ml per dish) consisting of 0.125 M NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1% Triton X-100, 3 trypsin inhibitory units per ml aprotinin (Calbiochem), 0.2 M Tris-HCl, pH 7.4. The cells were then homogenized with 20 strokes of a Dounce homogenizer (Kontis, pestle B) and clarified by centrifugation at 30,000g for 20 min. The resultant supernatant fluid was used for immunoprecipitations and affinity chromatography. Each sample contained 5×10^7 trichloroacetic acid-precipitable c.p.m. in a volume of 500 µl. All reactions were performed in 1.5-ml Eppendorf tubes. Lysates were first incubated for 1 h at 4 °C with 25 µl of staphylococcal protein A-Sepharose beads (as a 25% suspension in lysis buffer) that had been coated with rabbit anti-rat immunoglobulin serum (Cappel) and washed extensively (5 times, 15 volumes) with phosphate-buffered saline. This resin was removed by centrifugation and the preadsorbed lysate incubated for 1.5 h at 4 °C with no addition (lanes b, c, d, i) or after addition of 10 µl anti-transferrin receptor monoclonal antibody B3/25 (lane a), 2 µl anti-*ras* monoclonal Y13-238 (lane e), 20 µl normal rat serum (lane f), 2 µl of anti-*ras* monoclonal Y13-238 plus 20 µl normal rat serum (lane g), or 100 µg ml⁻¹ human diferric transferrin (lanes h, j). Following these incubations, 25 µl (as a 25% suspension in lysis buffer) of either uncoated protein A-Sepharose (lanes a, b) or protein A-Sepharose previously coated with an IgG fraction of rabbit anti-rat immunoglobulin serum (lane c), protein A-Sepharose previously coated with rabbit anti-rat immunoglobulin serum (lanes d-h), or transferrin-Sepharose (3 mg human diferric transferrin per ml resin) (lanes i, j) were added and the Eppendorf tubes tumbled for 1.5 h at 4 °C. The resins were washed by seven cycles of brief centrifugation and resuspension in 1 ml of cell lysis buffer. The final pellet was boiled in the sample buffer and analysed on 10–20% linear gradient polyacrylamide slab gel using the buffer system of Laemmli²². The gel was treated with 1 M sodium salicylate for fluorography before drying and exposure to Kodak X-ray film at -70 °C.

to lane *e*. When these lanes are compared in isolation, the presence of the transferrin receptor in the immunoprecipitate appears to be *ras*-specific.

Transferrin-laden normal rat serum is clearly an inappropriate control for monoclonal antibodies collected by salt precipitation from ascites fluid or from culture fluid of hybridoma cells grown in medium with or without 10% fetal bovine serum¹¹. Finkel and Cooper¹² also observed that transferrin added to the lysate eliminated the transferrin receptor band from anti-*ras* immune precipitates and proposed a transferrin-mediated conformational change in the receptor that disrupted the interaction with p21. They also suggested that a disruption of the p21-receptor complex by an anti-transferrin receptor monoclonal antibody was responsible for the fact that p21 was not co-precipitated by anti-receptor. The effect of transferrin and the asymmetrical co-precipitation are both explained more simply by the inadvertent affinity chromatography of the receptor demonstrated here. Loss of co-precipitation of the receptor on 'exhaustive precipitation' with anti-*ras*¹² may be due to the difference in the nature of the binding of the transferrin receptor to the resin in the absence and presence of anti-receptor antibodies. Only biologically active receptor can be bound by virtue of its affinity for the transferrin present on coated protein A-Sepharose. No such dependence on activity applies, however, in the binding of receptor by anti-receptor antibody. Thus, any loss of biological receptor activity during extraction or handling would result in transferrin receptor that could no longer become associated with coated protein A-Sepharose by affinity chromatography but which would still be precipitated with anti-receptor antibody. Partial inactivation of the receptor may also account for the high variability (5–50%) in the molar amounts of transferrin receptor apparently co-precipitated by anti-*ras*¹². It is unsurprising that several anti-*ras* monoclonal antibodies apparently co-precipitated the transferrin receptor¹² because the association of the receptor with the coated protein A-Sepharose is independent of the presence of the anti-*ras* (Fig. 1, lanes *b–d*). Using the methodologies of Finkel and Cooper¹² with normal rat serum as a control, addition of other monoclonal antibodies against any protein in the lysate would yield results suggesting that the transferrin receptor is co-precipitated specifically.

The results of Finkel and Cooper¹² have been reproduced here but additional controls support the lack of any molecular complex between p21 and the transferrin receptor. Although the 90,000 MW polypeptide observed¹² is the transferrin receptor, its presence in immunoprecipitates is demonstrated to be independent of anti-*ras*. I have shown here that it is due to inadvertent affinity chromatography resulting from the presence of transferrin on the coated protein A-Sepharose used in immunoprecipitation.

I thank E. Chang for her gift of T24 cells, M. Furth for the anti-*ras* monoclonal antibody, G. Ashwell for preparation of transferrin-Sepharose, and G. Ashwell, E. Chang and R. D. Klausner for discussions and comments.

Received 18 July; accepted 2 August 1984.

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FINKEL AND COOPER COMMENT—Since learning of the above results we have repeated our experiments with additional controls. We concur with Harford that our findings are explained by the artefact he describes and, therefore, do not indicate an interaction between transferrin receptor and *ras* proteins.

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Primary structure of human transferrin receptor deduced from the mRNA sequence

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In vertebrates all iron is taken up via the carrier protein transferrin¹. The carrier first binds its receptor and the receptor–ligand complex is then internalized via coated pits^{2–4}. The transferrin receptor is a transmembrane glycoprotein (apparent molecular weight (MW) 180,000) composed of two disulphide-bonded subunits (each of apparent MW 90,000)^{1,5,6}. It contains three N-linked glycan units and is post-translationally modified with both phosphate and fatty acyl groups^{5,7}. Here we have determined the nucleotide sequence of the coding region of the human transferrin receptor mRNA and from this deduced the amino acid sequence of the protein. The receptor does not contain an N-terminal signal peptide but there is a membrane-spanning segment 62 amino acids from the N-terminus. It therefore has a somewhat unusual configuration with a small N-terminal cytoplasmic domain and a C-terminal extracellular domain of 672 amino acids.

The clone pTR48 contains a 2-kilobase (kb) cDNA insert derived from the human transferrin mRNA⁸. Using this clone as a probe, a set of five overlapping cDNA clones was isolated containing, in total, 5 kb of sequence derived from the human transferrin receptor mRNA (Fig. 1). The mRNA is slightly over 5 kb long (ref. 9 and C.S., unpublished) thus this set of clones probably spans most of the length of the mRNA. We have used primer extension (Fig. 2) to show that the insert in clone pTR36 terminates only 20(±2) nucleotides from the 5' end of the mRNA. The nucleotide sequence of the inserts in the set of cDNA clones was determined and is presented in Fig. 3. There is an open reading frame which extends from nucleotide 33 to nucleotide 2,544. The first available initiation codon in this reading frame is at position 264 and translation using this codon

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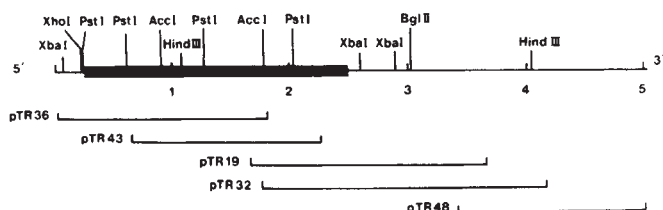
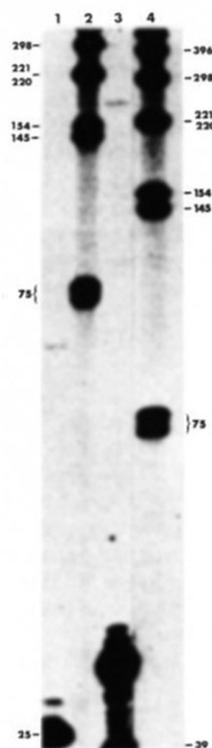


Fig. 1 Molecular cloning of human transferrin receptor cDNA sequences. The cDNA clone pTR48 was prepared using the orthodox 'hairpin cleavage' technique and shown by hybrid selection to contain human transferrin receptor sequences⁸. The complete nucleotide sequence of the cDNA clone was established using the dideoxy technique and the orientation of the insert was deduced by hybridization of cDNA to single-stranded DNA from one of the recombinant clones used in establishing the sequence. Analysis of the transferrin receptor mRNA by Northern transfer (data not shown) indicated that it was ~5 kb long, and the nucleotide sequence of the cDNA insert showed that it was derived from a noncoding region of the mRNA. An oligomer containing 18 nucleotides of sequence from very near the 5' end of the insert in pTR48 (given by Dr I. Stabinsky) was used to prime cDNA synthesis with ~5 µg of immune-enriched mRNA from MOLT-4 cells. This mRNA was derived from immune-selected polysomes in which transferrin receptor mRNA was estimated to constitute between 5 and 10% (by mass) of the mRNA population. Double-stranded (ds) DNA was prepared from the RNA-DNA hybrid using RNase H and the DNA polymerase I holoenzyme exactly as described by Gubler and Hoffman²¹. dsDNA >1,600 nucleotides long was selected by agarose gel electrophoresis and inserted into a pBR322-based vector, p2732K (J. Monahan, unpublished) by homopolymer tailing. The library of ~10,000 cDNA clones was screened by hybridization with a restriction fragment from near the 5' end of the insert in pTR48. Approximately 500 clones gave a positive signal and clones pTR19 and pTR32 were purified and used to screen for cDNA clones containing sequences more proximal to the 5' end of the mRNA. The entire set of overlapping cDNA clones shown was isolated in this way and yielded the indicated restriction map for a DNA copy of the human transferrin receptor mRNA. The map agrees precisely with that published recently by Kühn *et al.*⁹. Based on our estimate of the size of the mRNA, it seemed likely that the clone pTR36 would contain sequences from very near the 5' end of the mRNA and this was confirmed by primer extension (Fig. 2). (Given the method of construction of the clones, all of them might be expected to share a common 3' terminus—that is, the sequence of the oligonucleotide primer. However, only clone pTR32 has a terminus in the expected position. We believe this may be due to random priming because a very large excess of oligomer was used in the cDNA synthesis. This will not affect our sequence analysis because the region of overlap between clones is large and sequences across the termini were in each case established independently from another clone.)

generates a polypeptide 760 amino acids long. The deduced molecular weight is in very good agreement with that predicted by translation of the mRNA^{8,10} and the position of the presumptive transmembrane segment is precisely that expected (see below). Confirmation that this is the correct reading frame is provided by the amino acid sequence of two tryptic peptides (J. Downward, G. Scrace, R. Sutherland, M. Greaves and M. Waterfield, unpublished data) which are found in our predicted amino acid sequence at the positions indicated in Fig. 3 legend. The 5'-noncoding region of the mRNA is therefore somewhat longer than average (280 ± 2 nucleotides); it is also a little unusual in that there is an ATG trinucleotide upstream of the initiation codon. There are, however, precedents for both these features (reviewed in ref. 11) and the complete concordance with the expected protein structure suggests strongly that we have identified the correct N-terminus.

The 3'-noncoding region is also much longer than that of the average eukaryotic mRNA. We cannot determine the precise length of this region because pTR48, the 3'-proximal clone, does

Fig. 2 Analysis of the 5' terminus of the human transferrin receptor mRNA by primer extension. The conditions for the hybridization of the DNA primers to the RNAs and the extension reactions were as described by Devine *et al.*²² except that the temperature of the hybridization step was 55 °C. The first primer used was a single-stranded 25-nucleotide *HhaI*-*XbaI* fragment derived from within the 5'-noncoding region of the pTR36 transferrin receptor cDNA clone (Fig. 1) labelled at its 5' end with [γ -³²P]-ATP and T4 polynucleotide kinase. Lane 1 shows an extension product of 64 ± 2 nucleotides obtained with this primer and an RNA preparation immune-selected with a preparation of anti-transferrin receptor antibody as described by Schneider *et al.*⁸. Identical extension products were obtained using total placental RNA or size-selected (≥ 5 kb) placental RNA preparations (data not shown). The second primer was a single-stranded 39-nucleotide *XhoI*-*HaeIII* fragment derived from the beginning of the coding region of the transferrin receptor cDNA clone (Fig. 1), similarly labelled at its 5' terminus with [γ -³²P]-ATP and T4 polynucleotide kinase. Lane 3 shows the extension product of 250 ± 2 nucleotides obtained with this primer and a size-selected (≥ 5 kb) total placental RNA preparation. Identical extension products were obtained with immune-selected RNA or total placental RNA (data not shown). The size markers in lane 2 were loaded at the same time as the sample in lane 1. The size markers in lane 4 were loaded at the same time as the samples in lane 3. The lengths of the extended products obtained independently with these two primers demonstrate that the start of the human transferrin receptor mRNA is no more than 20 ± 2 nucleotides upstream from the start of the cloned cDNA.



not contain sequences derived from the poly(A) tail. It must, however, terminate very near to the end of the mRNA because the total length of the established nucleotide sequence closely approximates the length of the mRNA. Hence, the 3' noncoding region is >2,466 nucleotides long. Again, this is not without precedent and it is intriguing that the epidermal growth factor (EGF) receptor also displays such a feature¹². It is also interesting that the 3'-noncoding region contains several AATAAA sequence elements which are not used (Fig. 3).

Inspection of the receptor's predicted primary sequence reveals a stretch of 26 predominantly nonpolar amino acids with a sequence of nine hydrophobic residues in the centre (residues 63–88). When a hydropathicity plot (see Fig. 3 legend) was used to predict hydrophilic and membrane-embedded regions, this segment was the only convincing potential transmembrane segment. It would span, in an α -helical conformation, the 40 Å nonpolar part of the lipid bilayer (Fig. 4). The sequence, however, resides at the N-terminal end of the protein; no hydrophobic stretch is apparent at the C-terminal end. As the majority of the receptor is located at the external face of the bilayer, the receptor must be oriented with its N-terminus on the cytoplasmic side; this is in contrast to most membrane proteins which span the membrane close to the carboxy-terminus, exposing it on the cytoplasmic side (for review see ref. 13). A similar orientation has, however, also been found for several glycoproteins including the chicken asialoglycoprotein receptor, human erythrocyte band 3 protein and the murine and human Ia-associated invariant chain^{14–17}. The sequence of the putative transmembrane domain of the transferrin receptor permits multiple interactions within the lipid bilayer, as several residues are capable of forming hydrogen bonds. Also, a cysteine residue, which may

human EGF receptor, which comprises 542 amino acids¹².

The extracellular domain, which contains the transferrin binding site, comprises 648 amino acids. This domain contains three of the five possible sites for asparagine-linked glycosylation (Asn-X-Ser/Thr). As endoglycosidase digestion experiments have indicated that the receptor contains three N-linked glycan units, all three of these asparagines must be glycosylated.

Eight cysteine residues are distributed throughout the transferrin receptor sequence. Four cysteines are found in the region of the transbilayer peptide; two flank the peptide, one resides within the putative transbilayer sequence, and one is located close to the bilayer on the extracellular side. The remaining cysteines are present in two clusters in the extracellular domain (Fig. 4). It is unknown whether any of these cysteine residues form intrachain disulphide bridges. However, at least one must form the interchain disulphide bond(s) between the two 90,000-MW receptor subunits. Protein biochemical data suggest that the intersubunit disulphide bond(s) must lie close to the cell membrane, as trypsin treatment releases a non-disulphide-linked, 70,000-MW fragment from the cell surface⁵. It is unlikely that the cysteine (residue 62) on the cytoplasmic domain is disulphide-bonded due to the strong reducing environment of the cytoplasm. The cluster of three cysteines close to or in the bilayer are, therefore, the most likely candidates for making an interchain disulphide bond.

The amino-terminus of the transferrin receptor contains no obvious signal sequence. This observation is consistent with *in vitro* translation experiments in which the size of the primary translation product is indistinguishable from that of the endoglycosidase H-treated polypeptide synthesized in the presence of microsomes¹⁰. The lack of an N-terminal cleavable signal sequence is not unprecedented. No such signal sequence has been identified in the Ia-associated invariant polypeptide, which also has the reverse orientation in the plasma membrane¹⁷. Similarly, the secreted glycoprotein ovalbumin has been shown to contain an internal signal sequence²⁰. It is possible that the transbilayer sequence of the human transferrin receptor performs the dual function of acting as a signal sequence and of anchoring the protein in the plasma membrane.

When the amino acid sequence of the transferrin receptor was used in a search of the Dayhoff data base, no strong homology with any known protein was revealed. There is some homology with the EGF precursor and with the chicken transferrin precursor, but the degree of homology is very low (see Fig. 3 legend). Similarly, a search of a more specialized data base containing a comprehensive collection of oncogene sequences (G. Scrace, personal communication) revealed no strong homologies.

We thank Hiro Mahbubani for technical assistance, Michael Green for performing secondary structure analysis of the protein, and I. Stabinsky for providing the synthetic oligonucleotide described in the text. We also thank G. Scrace, J. Downward, R. Sutherland, M. Greaves and M. Waterfield for allowing us access to protein sequence data in advance of publication, and Rita Harris and Brenda Marriott for their help in preparing the manuscript. C.S. is on leave of absence from the Institute of Biochemistry, University of Trieste, Trieste 34127, Italy.

Received 30 July; accepted 7 September 1984.

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Three-dimensional structure of an antigenic mutant of the influenza virus haemagglutinin

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Antigenic variation in the haemagglutinin (HA) glycoprotein of influenza virus is associated with recurrent epidemics of respiratory disease in man (for review see ref. 1). We have examined the size of structural changes necessary to alter the antigenicity of HA by determining the three-dimensional structure of the HA from an antigenic mutant containing a single amino acid substitution which was selected by growth of virus in the presence of monoclonal antibodies. Here we present evidence that the simple addition of an amino acid side chain which results in only minor local distortions of the structure of the HA is sufficient structural alteration for a virus to escape neutralization by a monoclonal antibody. Our results also demonstrate that single amino acid substitutions can cause only local changes in the HA structure, verifying the assumption made in several studies to locate antigenic sites on the HA²⁻⁵ and other molecules^{6,7}, and indicate that proposals^{8,9} of large conformational changes to account for variations in HA antigenicity are unnecessary in this case. The structure of the variant antigen has independently been successfully predicted (M. Karplus, personal communication).

An antigenic variant, V3a, was selected by growing the parent (X31) virus¹⁰ in the presence of a neutralizing monoclonal antibody, HC3a, as described previously^{11,12}. Table 1 shows that while haemagglutination by the parent virus is inhibited by HC3a ascitic fluid at a dilution of 1:12,800, the variant virus V3a is not inhibited at a 1:100 dilution of the fluid. A similar result was obtained in enzyme-linked immunosorbent antibody binding assays, in which parent virus and V3a mutant virus bound antibodies at ascitic fluid dilutions of 1:100,000 and 1:100, respectively.

The amino acid sequence of the entire V3a HA was determined by sequencing the viral RNA using methods described previously^{13,17,18}; only one nucleotide change was observed, G to A, at nucleotide 514 which, when translated into amino acids, established that V3a has an aspartic acid at position 146 in the HA1 polypeptide instead of the glycine in the parental sequence¹⁹.

Isomorphous crystals of V3a were grown under conditions in which the X31 HA crystallizes¹⁴. (This has been observed for all of four single amino acid variants tested to date.) We collected 76,840 unique X-ray reflections to 3.0 Å resolution on 1° oscillation photographs recorded at 4°C by methods described previously¹⁵. Partially recorded reflections (≥50%) were included in the data set after adjustment by the post-refinement pro-

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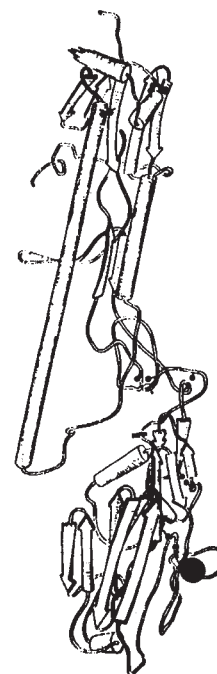


Fig. 1 A schematic diagram of a monomer of the haemagglutinin from the 1968 Hong Kong influenza virus¹⁵, showing the location of the single mutation, Gly to Asp 146.

cedure¹⁶. The symmetry *R* factor (see Fig. 2 legend) on fully recorded reflections is 0.11 and on all reflections more than half recorded, 0.14. The parent and variant structure factors were scaled together using a Wilson statistics curve (R scale = $(F_{\text{obs V3}} - F_{\text{obs X31}})/(F_{\text{obs X31}}) = 0.161$) and a difference Fourier was calculated to 3 Å resolution using phases from the refined model (see Fig. 2 legend for details).

The difference Fourier averaged about the molecular 3-fold axis shows nine peaks above the 5σ level. The largest peak is 12σ and is at the location expected for an aspartic acid side chain if it were added to Gly 146 (Fig. 2b); the next highest peak is <6σ. Similar results with fewer noise peaks were observed on a difference Fourier calculated with a shell of data from 10 to 6 Å resolution.

The coordinates of the aspartic acid were obtained from model building on a $2F_o - F_c$ map after refining native coordinates against V3a data. The O^δ of the carboxylate of Asp 146 appears within 3.0 Å of the N^ϵ of Arg 141, forming an apparent hydrogen bond. No other distortion of the external loop (amino acids 139–147) or of other features of the protein are observed, with the exception of a small (less than 1 Å) adjustment in the position of the side chain of arginine 141, presumably to accommodate the hydrogen bonding geometry required by the aspartic acid.

The structure of the variant HA suggests two possible mechanisms for the decreased affinity of the monoclonal antibody for the mutant HA: (1) The difference in the properties (shape, polarity and/or charge) of the aspartic acid and glycine side chains may directly affect the binding affinity. (2) The aspartic acid 146–arginine 141 interaction may stabilize the local structure of the site to which the monoclonal antibody binds, which would decrease the binding affinity if the antibody needed to locally distort or denature the site for tight binding. The former possibility corresponds to the classical 'static' view of an antibody–antigen complex, while the latter would apply if the free energy decrease on binding to a distorted site were greater than that required to distort the site. Present knowledge about the state of an antigen when 'seen' by immunogenic lymphocytes during the generation of an immune response and about the structure of an antigen–antibody complex leaves both possibilities feasible. In the case of V3a, the electrostatic charge of the Asp side chain would lead to unfavourable charge interactions (the burying of an unpaired charge) in complexes with antibodies against the parent (Gly).

Although multiple amino acid substitutions in the HA appear to be necessary for a virus to acquire the potential to cause an epidemic², single amino acid substitutions in the HA are sufficient for a virus to escape neutralization by monoclonal antibodies¹¹. The three-dimensional structure of the HA of an antigenic variant presented here indicates that the substitution of an aspartic acid for a glycine, without any significant changes in the positions of the atoms in the rest of the HA molecule, is a sufficient alteration to reduce by three orders of magnitude the binding of a monoclonal antibody. The absence of other structural changes in the variant HA, in turn, proves that the monoclonal antibody used to select this variant must bind directly to the region of the HA surface designated site A by Wiley *et al.*².

Since submitting this manuscript we have found that H. H.-L. Shih, J. Brady and M. Karplus (unpublished) have independently and with no prior knowledge of our results successfully predicted the structural changes in the mutant V3a, relative to the parent protein. Using a perturbation approach making use of the empirical energy function program CHARMM²³, they calculated a conformational potential energy surface²⁴ for the aspartic acid side chain in the presence of the rest of the protein. Energy refinement²³ of the low energy conformations on this surface yielded side chain torsion angles for Asp 146 with ±20° of the X-ray results. Further, the prediction of an adjustment

Table 1 Haemagglutination-inhibition reactions of X31 virus and antigenic mutant V3a

Virus	Monoclonal antibody				
	HC3a	HC31	HC101	HC83	HC263
X31	12,800	6,400	12,800	6,400	25,600
V3a	>100	6,400	12,800	6,400	25,600

Haemagglutinin-inhibition reactions were done by standard procedures. The values shown are the reciprocals of the highest dilutions of the antibodies at which haemagglutination was inhibited. The specificities of the different monoclonal antibodies are defined by the amino acid substitutions observed in antigenic variants of X31 virus that the individual antibodies select. Thus, antibody HC3a selects mutants with amino acid substitutions at residue 146; HC31 at residue 198; HC101 at residue 63; HC83 at residue 193; and HC263 at residue 158. All monoclonal antibodies tested, other than HC3a which was used to select V3a, react with X31 and V3a equally.

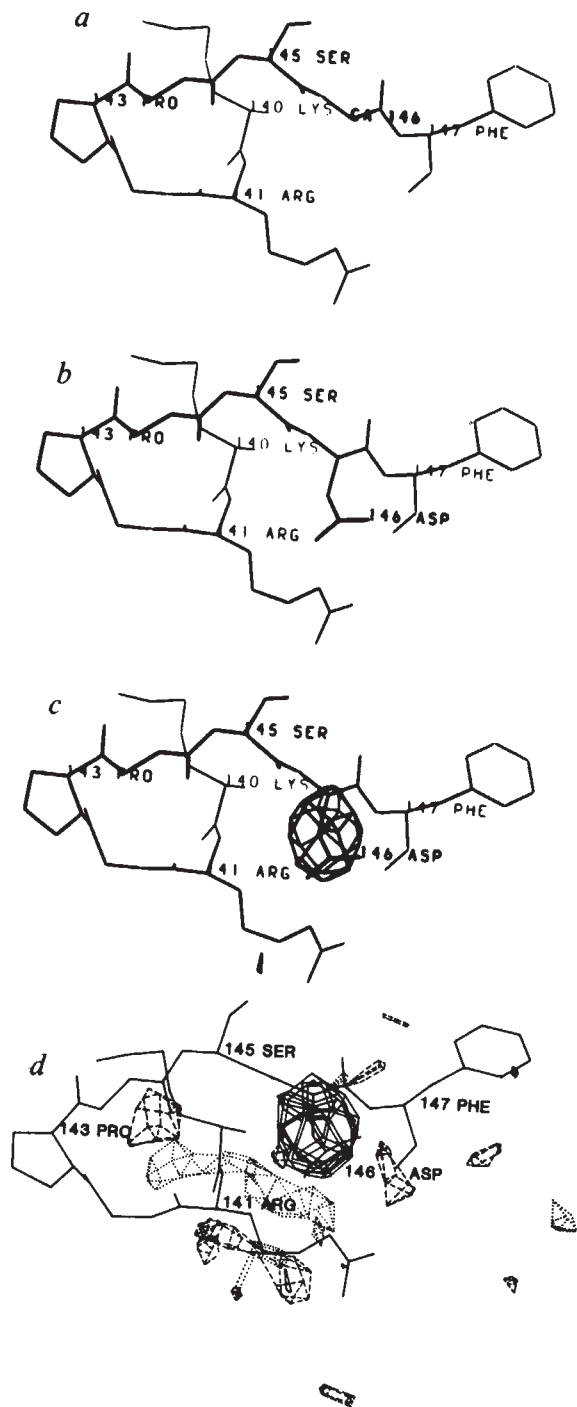


Fig. 2 *a*, Structure of the amino acid 140-147 loop which contains a glycine at position 146 in the parent HA. Atomic coordinates for the parent structure¹⁵ were obtained by model building (I. A. Wilson and D.C.W.) on a version of the BILDER program²⁰ modified by Robert Ladner. These were refined to an *R* factor of 0.26 after six cycles of restrained least-squares refinement²¹ where the contribution of the structure factor to the least-squares equation was evaluated by the gradient-curvature method on a difference map (ref. 22 and M. Lewis and D. Rees, personal communication). ($R = \sum |s_i I_{hi} - I_h| / \sum I_h$) where s_i = scale factor (including film temperature factor) for crystal i , and I_{hi} = intensity of reflection h of crystal i .) Exact 3-fold symmetry was imposed on the HA trimer in the crystallographic asymmetric unit by refining only a monomer's coordinates from 3-fold averaged difference maps. An overall *B* factor of 14 was used. Details of the refinement will be published elsewhere. *b*, The coordinates of the 140-147 loop of the antigenic variant V3a showing the location of the Asp (for Gly) substitution. Note that no conformational changes are observed between *a* and *b*. The V3a loop coordinates were obtained from model building on a $2F_0 - F_c$ map after refining the X31 coordinates (minus the loop) against the V3a data. *c*, The V3a difference map ($F_{\text{obs}} \text{ V3a} - F_{\text{obs}} \text{ X31}$) showing the 12σ peak at Asp 146. The only significant difference is at the position expected by the addition of the Asp 146 side chain instead of Gly 146 in the wild type. The difference Fourier was calculated using observed parent and variant structure factors with the refined phases described for *a*. *d*, The same as *c* with additional positive (dashed) and negative (dotted) contours at the noise level, 3σ . The interpretable 3σ peaks are a ridge of difference density indicating a small shift in the position of the side chain of Arg 141, which is within hydrogen bonding distance of Asp 146. (This figure was produced with an Evans and Sutherland PS-300 on the version of FRODO written by J. W. Pflugrath, M. A. Saper, B. Bush and A. Jones.)

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Errata

A general circulation model of water isotope cycles in the atmosphere

S. Joussaume, J. Jouzel & R. Sadourny
Nature **311**, 24-29 (1984)

IN this article, the order of the authors was incorrect. It appears correctly above.

Sequential late Cenozoic structural disruption of the northern Himalayan foredeep

D. W. Burbank & R. G. H. Reynolds
Nature **311**, 114-118 (1984)

THE above article was published with the name of the third author spelt incorrectly as Reynolds.

in the position of Arg 141 to improve the hydrogen bond to the carboxyl group of Asp 146 led to our finding its displacement in the experimental difference map. Details of the method and results will be given separately.

We thank David Stevens and Rose Gonsalves for assistance, Eugene Brown of Genetic Institute for synthesizing one of the oligonucleotide primers, and acknowledge support from NIH AI 13654 (D.C.W.). M.K. was the recipient of fellowship DRG-542 from the Damon Runyon-Walter Winchell Cancer Fund. We also thank Henry H.-L. Shih, John Brady and Martin Karplus for allowing us to quote from their unpublished results predicting the structure of the single-site mutant

Received 25 April; accepted 14 June 1984.

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Much ado about CO₂

John S. Perry

Man-Made Carbon Dioxide and Climatic Change: A Review of the Scientific Problems.

By P.S. Liss and A.J. Crane.

Geo Books, Regency House, 34 Duke Street, Norwich NR3 3AP, UK: 1983.

Pp.127. Hbk £8.50, \$17; pbk £3.95, \$7.80.

Carbon Dioxide — Emissions and Effects (Report No. ICTIS/TR18).

By Irene M. Smith.

IEA Coal Research, 14-15 Lower Grosvenor Place, London SW1W 0EX: 1982.

Pp.132. £10 (IEA countries), £20 (elsewhere).

Climate and Energy Systems: A Review of their Interactions.

By Jill Jäger.

Wiley: 1983. Pp.231. £19.95, \$39.95.

Our Threatened Climate: Ways of Averting the CO₂ Problem through Rational Energy Use.

By Wilfrid Bach.

Reidel: 1983. Pp.368. \$29, Dfl. 95, £24.25.

THE Niagara of writings on carbon dioxide, of which a sampling of the output over the past year or so forms the basis for this review, suggests that the gas is becoming an essential nutrient not only for green plants but for a large segment of the scientific community as well. Indeed, Roger Revelle — who, with Hans Suess, once termed man-made emissions of CO₂ a “geophysical experiment” — now wryly muses about the CO₂ “cottage industry”. What is all the fuss about?

The following is an attempt to compress a large bookcase into a paragraph. The Earth's carbon is distributed among crustal sediments, the ocean, a thin layer of living materials, a wisp of CO₂ in the air and millions of years' accumulation of fossilized organic material in the forms of coal, oil and gas. By burning the fossil matter and by converting carbon-rich forest to thinly vegetated agricultural land, we move carbon atoms into the atmosphere in the form of CO₂. Some of these atoms find new homes in the ocean or the living biota, but about half seem to stay in the air. Globally, we are injecting more than 5×10^9 tonnes of carbon into the environment each year, and the atmosphere's carbon dioxide concentration — now about 340 parts per million by volume — is increasing by about 1 ppm per year. This would be no great matter, except for the fact that CO₂ is a good radiator and absorber of thermal (infrared) radiation. Thus, increasing airborne CO₂ increases the effectiveness of the cool upper layers of the atmosphere as radiators and absorbers, and reduces the direct export of radiant energy from the Earth's surface to space. The lower atmosphere and surface must thus run at a higher temperature to make good the planet's energy accounts with the Sun and the cosmos. Altered heating and higher mean temperatures mean changes in climate, changes that must concern a world whose food supply depends on what Paul Waggoner has termed “the annual lottery

of the weather”. Is fossil fuel like tobacco, then: convenient and pleasant to burn, but with nasty long-term consequences?

To assess the issue, one must systematically explore every link in a long chain of processes. The marvellously complex nature of that chain has inspired increasing numbers of authors to try to sum up various findings and attempt a synthesis of the structure as a whole. The four volumes addressed here fall into this category. As in the classic *Rashomon* tale, each witness sees the scene through a filter of his own experience and background and tells a somewhat differently tinted story.

Liss and Crane, both associated with the electric power industry, follow a straightforward path through the major scientific questions attending CO₂ to their implications for future energy production from fossil sources. The authors' exposures of crucial physical processes such as ocean chemistry and radiation are clear, concise and accurate. Indeed, their analysis of the mechanics of the greenhouse effect is by a long mile the best short account in print. Irene Smith, writing for the coal and energy community, presents an objective and meticulously documented review that favours scientific results over methods and response strategies over unanswered questions. Jill Jäger takes a virtually orthogonal tack; after a notably complete essay on climate and carbon dioxide, she systematically assesses the connections between all aspects of energy production and climate. Her discussions of particulates, of solar and renewable energy sources, and of the effects of climate on the energy sector are particularly valuable. Finally, like his colleagues, Wilfrid Bach reviews the elements of the problem, but he never strays far from a path to its solution. Thus he focuses on the human society that is the source of CO₂ emissions, that must bear the consequences and that alone can devise means to cope with them. (In passing, it should be said that Jill Jäger

contributes to this collection not only as author of her own book and translator of Bach, but also as the prolific J. Williams who appears in the notably comprehensive bibliographies of all of the books.)

Despite their diverse approaches, the authors converge in their assessments. Indeed, their findings differ little from those of another recent volume about which I can hardly claim an unbiased view — *Changing Climate*, a report of the US National Academy of Sciences published in 1983.

● *How will the atmospheric CO₂ change?* This depends mostly on how much energy future populations will want and where they will get it. Forecasting approaches are diverse and predictions for the distant future span a wide range. Liss and Crane simply extrapolate constant growth rates, while Smith, Jäger and Bach review a number of forecasts, tending to favour the projections of the International Institute for Applied Systems Analysis. Jäger's discussion is particularly thorough, taking into account, for example, the logistical and sociopolitical obstacles to burning huge amounts of coal. Allowing for the uncertainties in the disposition of excess carbon by the ocean and atmosphere, all projections show rapid growth in airborne CO₂ as we move into the twenty-first century. Using an economic model in a Monte Carlo simulation, Nordhaus (in the NAS report) projected a probable doubling of airborne CO₂ in the third quarter of the century, with a broad band of uncertainty that comfortably envelopes the other studies.

● *What effects will there be on climate?* Numerical models of the climate system indicate that a doubled concentration of atmospheric CO₂, if maintained indefinitely, would produce a mean global warming of around 3°C. Regional changes in weather and precipitation would be more marked, but models cannot yet identify them reliably. It does seem that the poles would warm more than the tropics, and that mid-latitude summers would be longer, warmer and thus drier. Analogies with past and contemporary climate — carefully reviewed by Jäger — provide useful insights but no definite conclusions. Changes will be slowed and complicated by the heat buffering of the oceans, and other radiation-absorbing trace gases injected anthropogenically may add to the influence of CO₂. A particularly good discussion of the latter area is presented by Bach.

● *What are the implications of these changes?* In a warmer world, sea water will expand, glaciers will melt and sea level will rise (70 cm in a century according to the NAS study). Changes in weather will influence almost every aspect of human life — energy demand, agriculture, water resources, fisheries, settlement patterns, health and recreation, to follow Bach's exhaustive taxonomy. Especially troublesome are possible changes in

precipitation, evaporation and runoff that might affect the availability of water for irrigation. All of the authors see these changes as potentially serious, but only Bach agrees with a recent assessment by the US Environmental Protection Agency (*Can We Delay a Greenhouse Warming?*, which was also published last year) that the implications are unequivocally catastrophic.

● *What should we do?* If we side with all of the authors except Bach, giving weight to the huge uncertainties and the adaptive capabilities of life on Earth, then the wise course is to be alert for signs of nasty consequences (none is yet evident), to take sensible steps to reduce damage to the environment, and to develop a range of alternative options for energy supply and for living in a changing climate. If we share Bach's assessment then a much more active policy is called for. He proposes aggressive energy conservation measures, reforestation, soil conservation and the expeditious development of renewable energy resources. In the short term, the cautious and activist prescriptions may differ little in their practical effect, and by the end of the century, the NAS report proposes, we will know a lot more if we keep up the pace of research. In the meantime, the implementation of elements of Bach's prescription can only be to the good — fear of climate change, like fear of cancer, may lead us into healthier habits that could spare us from many other hazards.

Reading these books leads one to conclude that the CO₂ "cottage industry" is serving the public well by clearly illuminating the complexities of this web of scientific and social issues. Others in the business have different strengths. In a popular vein, John Gribbin's *Future Weather and the Greenhouse Effect* (Delacorte, 1982) has much to recommend it, if its more mystical elements are disregarded. Wilfrid Bach and colleagues

have given us two earlier and excellent reviews — *Man's Impact on Climate* (Elsevier, 1979) and *Carbon Dioxide: Current Views and Developments in Energy/Climate Research* (Reidel, 1983). Societal aspects were explored by W.W. Kellogg and R. Schwart in *Climate Change and Society* (Westview, 1981), while a detailed scientific analysis was orchestrated by Gordon J. MacDonald (*The Long-Term Impacts of Increasing Atmospheric Carbon Dioxide Levels*; Ballinger, 1982). A most comprehensive review, edited by William C. Clark, is *Carbon Dioxide Review: 1982* (Clarendon, 1982). And the NAS report, *Changing Climate*, a compendium of reviews, original contributions and synthesis, has already been mentioned.

The books of current vintage contribute much to this literature, and all can be recommended. Both Jäger and Bach are distinctive and important additions. Jäger's comprehensive view of climate through the prism of energy is unique and illuminating. Bach, though regrettably impeded by abominable computer typesetting, presents an impressively scholarly and exhaustive treatment. While his road leads to but one conclusion, it is broad and solidly built.

The next few years will bring further contributions. The US Department of Energy will soon release an assessment of the knowledge and conclusions emanating from its massive research programme, and an international effort is laying the groundwork for a 1985 intergovernmental conference. Thus, as was observed in the NAS report, while CO₂ may be a worrisome issue, it may also be a healthy stimulus which forces us to address seriously the long-term maintenance of our planetary home. □

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Cells: bags of water or blobs of jelly?

D.A.T. Dick

In Search of the Physical Basis of Life.

By Gilbert N. Ling.

Plenum: 1984. Pp. 791. \$79.50, £67.57.

QUANTITATIVE membrane theories, such as those of Boyle and Conway and the Hodgkin-Katz-Goldman equation, achieved spectacular success in the 1940s. These, along with Hodgkin and Keynes's demonstration of rapid potassium diffusion in the squid axon, induced a widespread view among physiologists that the cell is simply a membrane-bounded bag. In it, so the story ran, is a fairly homogeneous, aqueous soup of proteins and inorganic ions, the composition and electrical potential of which is controlled by the membrane. By contrast, a heterodox group of physiologists — led in particular by Troschin in Russia and by Ling in the United States — has maintained that the cell is essentially a blob of jelly, held together by controlled intermolecular forces between protein molecules to which water and the ionic constituents are variably bound. This view, called by Ling the association-induction theory, has been pursued to the extent of denying any significant role to the cell membrane.

Neither of these extreme stances is of course correct. In addition to the ample evidence for the function of the cell membrane, it is now known that the cellular matrix possesses a structure of microtubules and microfilaments (see for example *J. Cell Biol.* **99**, No. 1, Pt 2; 1984), that in some cells the distribution of ions (particularly sodium and potassium) is not uniform within the cell, and that at least some cellular water is bound. Thus there is sore need of an impartial review of alternative theories of the cell.

Dr Ling's book is divided into five sections, the first of which surveys concepts in cell physiology in a historical framework (here the most important chapter is the fifth which deals with the evidence for and against the membrane theory of the cell). The second section gives a full account of Ling's association-induction theory, particularly in relation to the control of the sodium, potassium and water contents of the cell. The third is largely devoted to the experimental work of Ling and his colleagues in support of the association-induction theory, while the fourth gives an account of mitochondria, muscle and epithelial transport, usually rejecting conventional physiological views and reinterpreting the data in terms of the association-induction hypothesis. The final section describes recent work on protein synthesis, growth and differentiation, and cancer, and offers speculative interpretations of these phenomena again



"Beluga or White Whale", from William Scoresby's *An Account of the Arctic Regions, with a History and Description of the Northern Whale-Fishery*, (1820). The illustration is reproduced from *Voyage into Substance: Art, Science, Nature, and the Illustrated Travel Account, 1760-1840*, by Barbara Maria Stafford, recently published by MIT Press. Price is \$39.95, £41.75.

based upon the association-induction hypothesis.

Dr Ling describes his book as "at once a monograph, a science history tract, and a textbook". A monograph, it certainly is, but it has little of the objective judgement and impartiality normally associated with a history or a textbook. For example there are 106 references to the work of Dr Ling while some quite distinguished cell physiologists are represented by a couple of citations only. A distinctive historical feature is a series of portraits, most of them of justly prominent figures but with some bizarre omissions (and inclusions); often one cannot help feeling that the space occupied by the photograph would have been better employed by a more adequate treatment of the work of the scientist portrayed. For example Ling's survey of

the sodium-potassium ATPase of the cell membrane, embracing the work of J.C. Skou, E.T. Dunham, I.M. Glynn, R.L. Post and S.L. Bonting, occupies but a page and a half.

Dr Ling appears to be constitutionally an advocate, rather than a judge, and he has set out to win his case by presenting every conceivable argument in support of his own position and giving the other side inadequate and unbalanced treatment. This is a pity. Since the 1950s majority views of the cell have moved significantly nearer to those of Dr Ling, but in this book he harms his own case by overstatement. What we need now is a judge, not an advocate. □

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From the laboratory to the clinic

W. F. Bynum

Science and Medicine in France: The Emergence of Experimental Physiology, 1790-1855.

By John E. Lesch.

Harvard University Press: 1984. Pp. 276. \$25, £22.

A SCIENTIST's proclaimed self-image may conceal as much as it reveals. Isaac Newton likened himself to a boy playing on the seashore, "diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me". This is a charming image, but one which hardly does justice to Newton's complex psychology, or to his belief that he had seen into the heart of things. François Magendie compared himself to "a rag-pricker: with my spiked stick in my hand and my basket on my back, I traverse the field of science and I gather what I find". Equally charming this, but equally inadequate as an account of Magendie's philosophy of discovery. Certainly he did not possess the philosophical sophistication of his pupil Claude Bernard (1813-1878), by whom this statement of Magendie was recorded and who rather liked to cast his teacher into the role of John the Baptist as foil to himself as Messiah. But Magendie (like Bernard) was concerned with the dialectic between basic science and clinical medicine, and it is this which is a central theme in John Lesch's book.

Magendie (1783-1855) was a child of the French Revolution, a product and a participant in the "hospital medicine" which, particularly after the reorganization of the Paris Medical School in 1794, came to dominate French medical education. This form of clinical medicine was firmly rooted in the hospital, where

medical students were to be based from the first day of their education and where they were to "read little, see much, do much". The high priests of hospital medicine — Corvisart, Laennec, Louis — stressed the importance of the local lesion as opposed to the generalized symptom, the lesion to be discovered by careful physical diagnosis (made more systematic after Corvisart popularized percussion and Laennec invented the stethoscope) and confirmed by autopsy. Every hospital physician was to be his own pathologist, permitting diseases such as phthisis, pneumonia and typhoid to be conceptualized in terms of more constant lesion than more variable symptom. For many doctors of the French school, the sciences of physics, chemistry, physiology and even microscopy were "accessory" rather than "basic" to the medical enterprise: accessory and potentially dangerous, for they encouraged speculation and system-building in advance of the "facts" demonstrable by pathological anatomy.

This aspect of "hospital medicine" has been elucidated in books such as E.H. Ackerknecht's *Medicine at the Paris Hospital, 1794-1848* (The Johns Hopkins University Press, 1967) and Michel Foucault's *Birth of the Clinic* (published in English translation by Tavistock in 1973). But while Lesch recognizes the cogency of their work, the value of his own lies in its patient examination of an equally rich tradition in early-nineteenth-century French medicine: a tradition which recognized the value of experimental science. He scrutinizes three sciences, chemistry, pharmacology and physiology, in each of which Magendie was a key figure.

A critical chemical tradition in French science dates back to the formation (in 1776) of the Société Royale de Médecine and to the rigorous attempt by prominent members of that Society, especially Antoine Fourcroy (1755-1809), to apply the newer analytical methods of chemistry to isolate and identify the active ingredients

of common medical preparations. From the turn of the century the discovery of plant alkaloids—including emetine, strychnine, codeine, quinine and morphine—had demonstrated the value of the "analytical method", and had provided Magendie with the justification for producing his own *Formulaire* (1821), a therapeutic manual based on the notion of pure substances rather than polypharmic preparations. In using the word "pharmacology" in 1805, J.B.G. Barbier (1776-1865) had insisted that the pharmacologist had to be both chemist (to identify the active substance) and physiologist (to elucidate its precise actions). It was particularly in the latter guise that Magendie pursued his experimental investigation of strychnine, prussic acid and ipecacuanha. His relations with the Paris hospitals were chequered, but various clinicians helped him in his researches; and, in 1830, he became director of a women's ward in the Hôtel Dieu, where he was able to conduct more systematic clinical trials.

Magendie's clinical work (some of it simply attempting to cope with the seriously ill, as in the 1832 cholera epidemic when this most gifted experimentalist of his generation treated 594 cholera victims between March and August) reminds us of the complexity of the relationship between science and medicine during the period. The interplay between the two can be seen in Magendie's concept of "pathological physiology", for much of his most famous research—on the cerebro-spinal fluid, on the functions of the posterior and anterior roots of the spinal nerves, on localization within the central nervous system—was laboratory based but clinically informed. Lesch establishes continuity between the methods and problems of the late Magendie and the early Bernard, just as he also sees important legacies for Magendie from the experimental work of Xavier Bichat (1771-1802).

Much of Lesch's monograph consists of rather detailed expositions of the experimental chemical, pharmacological and physiological results obtained by French medical scientists of Magendie's era. Some of this may be of specialist historical interest. But the general problem he addresses—what place has experimental science in medicine?—is one of great topicality. Our own answers may not be the same as those proposed in nineteenth-century France, but Lesch's book does illuminate an important historical moment in that continuing dialogue. □

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●Subscription rates were omitted from the review of the journal *Molecular Biology & Medicine* (Nature 311, 318; 1984). Publisher is Academic Press, and rates for 1985 are: institutional £60 (UK), \$100 (elsewhere); personal £30 (UK), \$50 (elsewhere).

Meeting of minds in a magnetic field

Robert S. Coe

Geomagnetism of Baked Clays and Recent Sediments.

Edited by K.M. Creer, P. Tucholka and C.E. Barton.

Elsevier Scientific: 1983. Pp. 324. Dfl. 125, \$53.25.

K.M. Creer and his co-editors frankly confess to holding "mixed views about the publication of symposium proceedings". Such pessimism was misplaced; to my mind, they have succeeded admirably in avoiding the pitfalls that doom most books of this genre to a limited readership and transient life.

Several factors have contributed to the success of this volume. First, the subject of geomagnetism has entered one of those periods in which the various sub-disciplines are bubbling with excitement and appear to be converging on common (but as yet dimly perceived) ground. Satellites such as MAGSAT have revolutionized the business of global measurement and description of the present geomagnetic field, cryogenic magnetometers have greatly increased the rate that data about the ancient geomagnetic field can be obtained from weakly magnetized rocks, and computers have immeasurably improved our ability to store and manipulate data and perform the calculations required for increasingly sophisticated models of geodynamos. Geomagnetists are now talking to palaeomagnetists; theory is meeting observation and experiment.

Secondly, the Fourth Assembly of the International Association of Geomagnetism and Aeronomy held in 1981 in Edinburgh—including the Symposium on Time Scales of Secular Variation, from which the core of papers published in this volume is drawn—was graced with an unusual number of truly fine contributions, and came at a time when the subject is manageable in scope and sorely in need of review. The main reason, however, that this book is definitely more than just another symposium volume lies in the clear effort taken by the editors to achieve a logical organization, adequate coverage and balance, and a semblance of uniformity of style.

The central theme is the behaviour of the geomagnetic field over the past several tens of thousands of years, as deduced from the remanent magnetization of baked clays and lake sediments. The 33 contributions (by 40 authors) are grouped into four chapters, the first of which contains brief and often interesting critiques of the most relevant magnetization processes, including thermoremanent magnetization, detrital remanent magnetization and shear

remanent magnetization. The second consists of similar treatments of selected dating techniques (palynology, radio-carbon, tree-rings and varves, lead-210 and thermoluminescence).

The third chapter is the longest and most highly developed. It is distinguished by a particularly clear and informative review of techniques for archaeointensity determination by M.J. Aitken; by summaries of archaeomagnetic results up to 1981 for Greece, Bulgaria and Yugoslavia, Egypt, the Near East, the USSR, China, Japan, the United States, Peru and Australia; and by a perceptive analysis of global intensities for the past 50,000 years by M.W. McElhinny. The final chapter consists of presentations of palaeomagnetic data—many of which show encouraging consistency—of lake sediments from Europe, North America, Argentina and Australia, and cave sediments from Europe, as well as reviews of mathematical techniques for analysis of directional data as a function of depth or time. Throughout the book, the various topics are woven together by means of introductory sections and occasional

summary discussions within each chapter. An "Epilogue" by the editors, containing their own analysis, interpretation and conclusions, also helps to unify the book and is a stimulating contribution in its own right.

One can always find things to complain about. I found myself wishing at times that the coverage were more encyclopaedic, and resolving to get my eyes examined because of the small camera-ready type-face with commas that are almost indistinguishable from full points. While it is impossible for a symposium volume to have the same intellectual impact as a book written by one or a few excellent scientists who are also excellent writers, this is about as close as you can get; newcomers can read it as a detailed and relatively unbiased introduction to the field, and practising specialists will find it a handy reference for quite some years. Simply put, it is one of the most effective multi-author works that I have encountered in the earth sciences. □

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Instruments of change

Alan Braithwaite

Microcomputers and Laboratory Instrumentation.

By David J. Malcolm-Lawes.

Plenum: 1984. Pp. 246. \$35, £29.75.

MOST scientists and technologists are now faced with the challenge of the microprocessor chip. It is present in all types of instrumentation, to control the mechanics and optimize parameters, and also to collect and process data. It is still possible to lead the quiet life of an alchemist, but the march of progress means that sooner or later almost everyone in a laboratory will have to wrestle with a new language—the language of the computer—and with sophisticated instruments employing arrays of chips.

Programming languages are not too difficult to learn. They use an understandable syntax, and there are numerous books on the topic. The hardware presents more of a problem but, surprisingly, there are few accounts of the subject as it applies to scientific instrumentation and intended for the practising scientist. David Malcolm-Lawes has written such a text. It sets out to present in clear, understandable terms the overall concept of microcomputer systems, microprocessors and various logic chips incorporated into digital and interfacing circuitry. Further, many computers are used for collection of data from detectors

and transducers, and also to control instrument functions, switches, relays, heaters and so on, and these principles too are explained both in general terms and in more detail, including the logic required to buffer and interface digital signal buses and interconnect equipment. For the more enterprising readers there is also a chapter on system design concepts and the construction of interfaces and microcomputer equipment.

It is quite possible to read the book selectively to obtain an overview, but the main purpose is to cover all the necessary background concepts relating to the nature and handling of instrument and transducer signals, microcomputer systems and interfacing concepts. Overall, the writing reflects the teaching experience of the author, as does the inclusion of a large number of diagrams to supplement the text—in this subject in particular, a picture is indeed worth a thousand words.

The net result is a well-presented, readable book which provides a sound introduction to microcomputers in the laboratory and also to the principles and concepts of microprocessor systems and interfacing. Scientists and technologists seeking to get to grips with new technological developments, and students for whom laboratory instrumentation is part of their course, will find it very helpful. My only plea is that the publishers should consider issuing a paperback edition; I think they would find it worth their while. □

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Mathematics

Calculus: Basic Concepts and Applications. By R.A. ROSENBAUM and G. PHILIP JOHNSON. Cambridge University Press: 1984. Pp.422. ISBN 0-521-25012-9. £12.50, \$24.95.

Mathematical Essays in Honor of Su Buchin. C.C. HSIUNG (ed.). World Scientific: 1983. Pp.276. ISBN 9971-950-98-7. £26.35.

Multifaceted Modelling and Discrete Event Simulation. By BERNARD P. ZEIGLER. Academic: 1984. Pp.372. ISBN 0-12-778450-0. £25.50; \$42.50.

Nonparametric Functional Estimation. By B.L.S. PRAKASA RAO. Academic: 1983. Pp.522. ISBN 0-12-564020-X. \$70.

The Umbral Calculus. By STEVEN ROMAN. Academic: 1984. Pp.193. ISBN 0-12-594380-6. \$35.

Unified Integration. By E.J. McSHANE. Academic: 1983. Pp.607. ISBN 0-12-486260-8. \$55.

Astronomy

Astrodynamics 1983. Advances in the Astronautical Sciences, Vol. 54 Parts I & II. T.G. TSENG *et al.* (eds). American Astronautical Society: 1984. Pp.653 & 1,330. Hbk ISBN 0-87703-190-7; 0-87703-190-8; pbk ISBN 0-87703-191-6; 0-87703-191-6. Np.

Connected Components in Binary Images: The Detection Problem. By CHRISTIAN RONSE and PIERRE A. DEVIJVER. Wiley: 1984. Pp.165. ISBN 0-86380-016-5. £24.

Interstellar Chemistry. By W.W. DULEY and D.A. WILLIAMS. Academic: 1984. Pp.264. ISBN 0-12-223360-3. £29, \$45.

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Technology

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Flexible Assembly Systems. Assembly by Robots and Computerized Integrated Systems. By A.E. OWEN. Plenum: 1984. Pp.231. ISBN 0-306-41527-5. Np.

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Chemistry

Advanced Organic Chemistry 2nd Edn. Part A: Structure and Mechanisms. By FRANCIS A. CAREY and RICHARD J. SUNDBERG. Plenum: 1984. Pp.726. Hbk ISBN 0-306-41087-7; pbk ISBN 0-306-41198-9.

Analysis Using Glass Electrodes. By PETER W. LINDER, RALPH G. TORRINGTON and DAVID R. WILLIAMS. Open University Press: 1984. Pp.148. ISBN 0-335-10420-7. £20.

75th Jubilee Conference on Helium — 4. J.G.M. ARMITAGE (ed.). Wiley: 1983. Pp.211. ISBN 9971-966-23-9. £20.75.

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Organometallic Chemistry, Vol.12. F.G.A. STONE and E.W. ABEL (eds). Royal Society of Chemistry: 1984. Pp.498. ISBN 0-85186-601-8. £82.

Reactions of Small Transient Species: Kinetics and Energetics. A. FONTIJN and M.A.A. CLYNE (eds). Academic: 1983. Pp.488. ISBN 0-12-262040-2. £49.80, \$89.

Room Temperature Phosphorimetry for Chemical Analysis. By TUAN VO-DINH. Wiley: 1984. Pp.304. ISBN 0-471-87884-7. £47.50.

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Surfactants in Solution, Vols 1 & 2. K.L. MITTAL and B. LINDMAN (eds). Plenum: 1984. Pp.705, 1,432. ISBN 0-306-41483-X; 0-306-41484-8. Np.

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Physics

Advances in Liquid Crystals, Vol. 6. GLENN H. BROWN (ed.). Academic: 1983. Pp.268. ISBN 0-12-025006-3. \$80.

Classical Mechanics: A Course of Lectures. By A.K. RAYCHAUDHURI. Oxford University Press: 1984. Pp.210. ISBN 0-19-561343-0. £7.50.

The Creation of Quantum Mechanics and the Bohr-Pauli Dialogue. Studies in the History of Modern Science 14. Reidel: 1984. ISBN 90-2771648-X. \$34.50.

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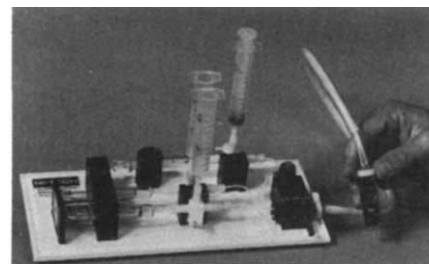
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● A new compact radioisotope camera for the visualization of radioactive distributions of tritium and carbon-14 labelled compounds on TLC and paper chromatograms is now being produced by Birchover Instruments Ltd of Letchworth. The instrument uses a high resolution scanning spark chamber detector to produce a Polaroid print approximately 1/3 full size of the radioactive separations with exposure times of between 20 to 120 min, reducing considerably the evaluation time as compared with autoradiography which would require 3 to 21 days for a comparable result. A print projector for projecting the Polaroid print back so that radioactive areas can be quickly marked out on to the original radiochromatogram is an integral part of the bench top instrument.

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● Imperial Laboratories (Europe) Ltd. of Salisbury has developed a serum-free lymphocyte culture medium for cytogenetics. This medium, Iscove's modification of Dulbecco's MEM, has the folic acid level reduced from the normal 4.00mg to 0.01mg per litre. Imperial Laboratories will undertake the production of cell culture media to customers' specifications in addition to supplying their range of standard media and sera. The medium was developed in conjunction with the Wessex Regional Cytogenetics Unit at Salisbury General Hospital.

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• Ten purified homologues of tunicamycin are now available from Boehringer Mannheim Biochemicals. Each homologue exhibits a different degree of inhibition of glycosylation and overall protein synthesis. Thus, using a purified homologue rather than a standard commercial preparation of tunicamycin (mixtures of these homologues), achieves a much greater precision of inhibition. The levels of inhibition of protein synthesis by tunicamycin homologues and glycosylation are dependent on the system and growth conditions. Selection of the homologue with the desired inhibitory effects will be largely empirical.

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• From LKB Instruments, Inc., comes a new pamphlet on the bioluminescent assay of NADH and NADPH. Application Note 506 offers a useful summary of the current state of the art, plus two generalized bioluminescent assay protocols, and a selected bibliography of applications in enzyme and metabolite analysis. The light produced by these luminescence methods is from two purified forms of bacterial luciferase specific for either NADH or NADPH. Measurement of light is simple as the quick flash problem has been solved by the monitoring reagents. In the applications section 14 dehydrogenases and other enzyme references are listed.

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ADVERTISEMENT

How to avoid the 19 critical mistakes in buying fermentation equipment

Make one mistake and you can have problems that include inadequate process control, permanent operator inconvenience and costly downtime.

Now NBS has published a comprehensive review of the problems and pitfalls that await the unwary buyer of industrial fermentors — and some very practical suggestions to help avoid them.

Called *19 Critical Mistakes Made in Buying Fermentation Equipment and What You Can Do to Avoid Them*, this practical guide is available without charge.

Send for your free copy today. New Brunswick Scientific Co., Inc., P.O. Box 986, Edison, NJ 08818. (201) 287-1200 or (800) 631-5417.



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**19
CRITICAL
MISTAKES**
made in buying
fermentation
equipment
and
what you
can do to
avoid
them

Avoid These 19 Critical Mistakes When Buying Fermentation Equipment

1. Lack of a Recirculating System

Recirculation is an important feature in any fermentation system. It ensures that the culture is well mixed and that the temperature is uniform throughout the reactor. A lack of recirculation can lead to poor yields and even to the death of the culture.

The design of the recirculating system is critical. It should be able to handle the volume of culture being fermented and should be able to operate at the required pressure and temperature. A lack of recirculation can lead to poor yields and even to the death of the culture.

2. Failure to Provide Pressure Regulators and Filters

Pressure regulators and filters are essential components of any fermentation system. They ensure that the culture is exposed to the correct pressure and that any contaminants are removed from the gas supply. A lack of pressure regulators and filters can lead to poor yields and even to the death of the culture.



• The Bio-Rad Bio-Gel TSK SP-5PW cation exchange HPLC column offers the advantages of high resolution, high sample loads, and high recoveries of biological activity in protein and peptide analysis. Unlike silica-based ion-exchange HPLC columns, the SP-5PW column, with its resin based packing, permits a pH operating range of 2–12. This wide pH range gives flexibility in optimizing protein separations and simplifies column cleanup procedures. Either acid (0.1M HCl) or base (0.1M NaOH) washes may be used to clean the column. The 1,000 Å pore size of the SP-5PW matrix enables the column to separate proteins with molecular weights as high as 10^6 . The column has a capacity of 1–5 mg for the protein of interest.

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A new system for applying samples for electrophoresis—the Pratiga from Shandon

• The Shandon Pratiga system is a new application system for electrophoresis, designed for separations on cellulose acetate membranes including serum proteins, haemoglobins, lipoproteins and isoenzymes. The Pratiga system performs up to 48 separations in one run using 'DispoCards' which 'stroke' the sample onto the applicator ensuring even deposition everytime. The sample is applied in a long narrow rectangle, regarded as the best geometric form for optimum scanning and sample evaluation. There are no capillary effects or globules of sample on the side of the applicator avoiding the inherent design faults of conventional wire-type applicators.

Circle No. 112 on Reader Service Card.

• World Precision Instrument Inc.'s new S7000A modular microelectrode system combines proven technology with latest advances. Features include ultra-stability, low noise, and ± 100 volt compliance. Mainframes are available in either single or four channel designs, and modules are available for iontophoresis, electrometer, and a voltage/patch clamp. Each module is supplied with one probe of the end-user's choice.

Circle No. 113 on Reader Service Card.

● Tissue, organs, tumours and coatings can be rapidly disintegrated using Heat Systems-Ultrasonics' ultrasonic debrider, and the resulting homogenate is removed in the same operation. Liquids such as water, saline, acid, or solvents travel down the centre of the needle and flood the tip. Intense ultrasonic energy disrupts any solid matter near the tip, even bone. Simultaneously, the fluidized mass or tissue is sucked through the needle and exits at the rear of the probe. Both ultrasonic energy and liquid flow are controllable. The probe is 6 inches long and available in three tip diameters: 3/8 in, 1/8 in, and 0.010 in.

Circle No. 114 on Reader Service Card.

● A new 5 mm Radial-Pak cartridge with Waters Nova Pak C₁₈ packing for high sensitivity HPLC analyses is now on the market. The cartridges allow HPLC analyses with the efficiency (100,000 plates per metre) to separate acids, neutrals and amines within the same mixture. Used with Waters Radial Compression Separation Systems (RCSS), Waters Radial-Pak cartridges are low cost, flexible-wall HPLC 'columns'. When radially compressed in a RCSS module, the cartridge provides a stable column bed which results in longer life, typically three times longer than a conventional steel column. Nova Pak C₁₈ is a 4- μ m spherical packing featuring an exclusive bonding end-capping process which provides a stable and durable stationary phase for wide selectivity of all compound classes.

Circle No. 115 on Reader Service Card.

● The Miniultrasart from Sartorius is a self-contained ultrafiltration module with a 20,000 molecular weight cutoff membrane. The Miniultrasart offers high flux rates and long service life and is specially suited for depyrogenation of water and other liquids, and concentration/fractionation of macromolecules.

Circle No. 116 on Reader Service Card.

● A new bulletin from Bio-Rad describes 7 carbohydrate analysis HPLC columns, each specially tailored to separate a specific class of carbohydrate, and discusses ways to optimize separations with each column. Topics discussed include selecting the proper column, solvent selection, organic modifier concentrations, and optimizing column temperature and flow rate.

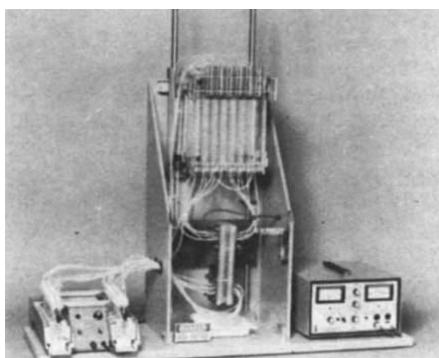
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ADVERTISEMENT

Liposomes

A new laboratory instrument (LIOPREP®-GD-1) is available for the reproducible preparation of extremely homogeneous, unilamellar liposomes of controllable size. The instrument permits the simultaneous use of five different lipid mixtures and provides extensive versatility. Applications range from membrane reconstitution, remodelling of cellular functions, liposome drug delivery systems, microanalysis of drugs and metabolites, to antibody targeting, clinical diagnostic assays, and genetic engineering.

For details write to:
DIANORM-Geräte, P.O. Box 126, D-8 München 65, FRG.



The Isoprep isoelectric focusing apparatus.

● The latest LC 5001 amino acid analyser, available from Astra Scientific International, features a high pressure fluid delivery/sample injection system for efficient column packings and an automatic reaction coil flush for emergency and scheduled instrument shut-downs. A microprocessor controls the entire analytical protocol with eight user-defined programs. Chromatographic separations require only 45 min for protein hydrolysates and 95 min for biological fluids. An adjustable temperature-gradient profile fine tunes chromatographic separations, and standard features include dual HPLC pumps, a refrigerated compartment for storage of samples, eluents, and reagent, dual-channel photometer, and dual-channel recorder.

Circle No. 118 on Reader Service Card.

● A new line of plastic, disposable serological pipettes featuring continuous dispensing tips for accurate fluid delivery is now available from Bellco Biotechnology. Made of high clarity polystyrene with crisp, fine line graduations and large numbering for easier reading, the pipettes' pulled tips provide smooth internal wall surfaces which cannot trap and hold back fluid when dispensing. They are calibrated for accurate delivery with dispensing accuracy rated within $\pm 2\%$. Bellco plastic serological pipettes are compatible with all standard pipetting devices, and are available in four sizes to suit most laboratory needs. The four sizes are: 1 ml with 0.01 ml graduations; 2 ml with 0.01 ml graduations; 5 ml with 0.1 ml graduations; and 10 ml with 0.1 ml graduations. All are provided with cotton plugs and packed in sterile individual wrappers, or sterile canister packs.

Circle No. 119 on Reader Service Card.

● The Hamilton Microlab 1000 diluter/dispenser is capable of carrying out standard curves with up to 10 diluting steps in one program. In addition, it has the following optional functions: serial diluting, serial pipetting, multiple pick-up, serum distribution, diluter function, double diluter, dispensing and repetitive dispensing. Up to 50 programs can be stored and retrieved. On the sample side syringes of 50 μ l to 5 ml and on the diluting side from 50 μ l up to as much as 25 ml can be used as required.

Circle No. 120 on Reader Service Card.

● The Medical Products Division of Ionics, Incorporated has introduced a new recirculating isoelectric focusing system for separation of proteins. IsoPrep systems can be used to separate synthesized peptides and enzymes on a preparative scale and is scalable for production of genetically engineered proteins. The IsoPrep system separates proteins of close pI's under the influence of applied d.c. electrical current. It uses porous membranes to form multiple compartments, each with a different pH. The membranes prevent convection but allow the proteins to migrate freely until they reach a channel with pH equal to their respective isoelectric points and concentrate there to be collected.

Circle No. 121 on Reader Service Card.

● The Nytran membrane from Schleicher & Schuell is a modified, positively charged nylon-66 solid support medium for use in nucleic acid and protein transfers. The new membrane offers a high binding capacity that allows for complete immobilization of all samples. It gives low backgrounds and has the ability to bind nucleic acids at low molecular weight, making it especially suited for electrophoretic transfers. It is also suitable where re-probing is necessary.

Circle No. 122 on Reader Service Card.

● Tresyl-activated Sepharose 4B is a pre-activated affinity medium for the immobilization of small ligands and proteins. The new medium can accomplish easy and reproducible coupling through both thiol and amino functional groups. Coupling capacity is affected only by the nature of the ligand, the temperature, and the pH. Since the active coupling groups of this affinity medium are stable to hydrolysis, unused swollen gel can be stored for at least one month. Tresyl-activated Sepharose 4B, from Pharmacia is supplied in 15g pack sizes which yields approximately 60ml of gel.

Circle No. 123 on Reader Service Card.

● Dionex's new amino acid analysis systems are claimed to bring more speed, reliability, and simplicity to amino acid analysis. Based on a new pellicular cation exchange resin, it combines the specificity of ion-exchange chromatography with the speed and simplicity of reversed phase HPLC methods. Conventional cation exchange resins require long separation and equilibration times (60-90 min total analysis time). Reversed phase methods, while offering fast run times, suffer from sample matrix interferences, instability of pre-column derivatives and short column life. Dionex's new cation resin operates at ambient temperature in a completely non-metallic system using very dilute, non-citrate eluents. Hydrolysate analyses are completed in less than 30 min.

Circle No. 124 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

Awards

The **Medical Research Council UK** annually awards Senior Research Leave Fellowships and Senior Clinical and Non-Clinical Fellowships. For further details contact the Grants Section, MRC, 20 Park Crescent, London W1N 4AL, UK.

Applications are invited by the **International Foundation for the Promotion of Nutrition Research and Nutrition Education (ISFE)**, Zug, Switzerland for the 1985 ISFE Prize on research into new approaches to the prevention and/or treatment of obesity. Conditions of entry for the Sfr. 15,000 award are obtainable from the President of the Foundation, Professor Dr J.C. Somogyi, Nideldbadstrasse 82, 8803, Rüschlikon, Switzerland.

The **International Association of Gerontology** has announced that the 2nd Sandoz Prize for Gerontological Research (worth 20,000 SFr) will be presented in July 1985. Further information from Dr Leo Abisch, International Association of Gerontology "Sandoz Prize for Gerontological Research", Box 4043, CH-4002 Basle, Switzerland.

The **Scientific Council of the International Centre for Theoretical Physics**, Trieste, Italy is again offering an annual prize of US \$1,000. For further information contact the 1984/1985 Prize Committee, International Centre for Theoretical Physics, PO Box 586, 34100 Trieste, Italy.

For details of the International Research Awards offered by the **World Population Council** on Determinants of Fertility in Developing Countries, contact the Program Manager, The Population Council, 1 Dag Hammarskjöld Plaza, New York, New York 10017, USA.

The **National Science Foundation (US)** Alan T. Waterman Award Committee has issued a call for nominations of candidates for its tenth annual award intended to give recognition to outstanding US researchers under 35 in any field of science, mathematics or engineering. Additional information from the Executive Secretary for the Committee, Mrs Lois J. Hamaty, NSF, Washington D.C. 20550, USA.

Appointments

Mr Roger Courtney has been appointed as head of the Technology and Innovation Directorate of the Energy Efficiency Office the Department of Energy, UK.

Dr K.J. Treharne, Head of the Plant Sciences Division at the Long Ashton Research Station, Bristol University, is now to take over as Director; he has also been appointed to a Chair and the Headship of the Department of Agriculture and Horticulture within the University.

Alan Watson, Reader in Particle Cosmic Physics in the Department of Physics and Dean of the Faculty of Science at Leeds

University, has been appointed a Professor of Physics at the University.

Mr Don Metz, currently Deputy Secretary with the Department of Territories and Local Government in Canberra, Australia, has been appointed as the new Director General of the Commonwealth Agricultural Bureaux — the first Director not to be resident in the United Kingdom. **Ms Gill Glass** has been appointed Managing Director of Bachem UK, the British branch of Bachem Inc., USA.

Mr F. Edward Gustafson and **Mr William F. Hendershot** have been elected as vice-presidents of Miles Laboratories Inc., Indiana, USA.

Dr. Muroi, currently president of the University Corporation for Atmospheric Research in Boulder, Colorado, has been nominated to a six-year term as a member of the National Science Board, Washington D.C., USA.

Dr. Charles Elachi has been named manager of the Earth and Space Sciences Division of the Jet Propulsion Laboratory, NASA, California.

Meetings

22-26 October, **European Wind Energy Conference and Exhibition**, Hamburg, FRG (H.S. Stephens & Associates, European Commercial and Technical Conferences, Agricultural House, 55 Goldington Road, Bedford MK40 3LS, UK).

29 October-1 November, **Second International Conference on Computers in Science**, Washington D.C., USA (Computers in Science, Scherago Associates, Inc., 1515 Broadway, New York, New York 10036, USA).

14 November, **Meeting on High Complexity Displays**, London (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX, UK).

15 November, **Seminar on Plan for Computer Integrated Manufacturing**, London, UK (The Manager — Conferences and Exhibitions, The Institution of Production Engineers, Rochester House, 66 Little Ealing Lane, London, W5 4XX, UK).

15-17 November, **3rd Epileptology Meeting — The Epileptic and His Family**, Coimbra, Portugal (Dra. Margarida Santiago, Clinica Neurológica, Bloco Hospital de Celas, H.U.C. 3000 Coimbra, Portugal).

20 November, **Paper on Technological Change — The Effect on People at Work**, London (The Manager — Conferences and Exhibition, The Institution of Production Engineers, Rochester House, 66 Little Ealing Lane, London W5 4XX, UK).

22 November, **Hockley Memorial Lecture on Communications and Propaganda**, London, UK (Secretary, Institute of Scientific and Technical Communicators, 17 Bluebridge Avenue, Brookmans Park, Hatfield, Hertfordshire AL7 9RY).

26-28 November, **2nd International Conference and Exhibition on New Technology and the Food and Drink Industries**, Bristol, UK (Mr David Jones, New Technology & the Food & Drink Industries Conference, Brintex Limited, 178-202 Great Portland Street, London W1N 6NH, UK).

27-28 November, **11th Mammalian Molecular and Biochemical Genetics Workshop**, London, UK (Dr G. Bullfield, Genetics Group, AFRC Poultry Research Centre, Roslin, Midlothian EH25 9PS, UK).

4 December, **International Conference on Hybridomes**, Paris, France. (INNOBIO, Scientific Association for Biotechnology Advancement, 26 Rue Guynemer, 75006 Paris, France).

17-19 December, **Annual Conference of the Society for Research into Higher Education**, London (SRHE, University of Guildford, Surrey GU2 5XH, UK).

3-5 January, **2nd International Conference on Clinical and Basic Factors Influencing Bone Growth**, Los Angeles, USA (Prof A.D. Dixon, School of Dentistry and Medicine, Dental Research Institute, Center for Health Sciences, University of California Los Angeles, Los Angeles, California 90024, USA).

12-16 January, **Keystone Summit on Allergy, Immunology, Pulmonology, and ENT**, Colorado, USA (Ms Helga Cole, National Jewish Hospital and Research Center/National Asthma Center, 3800 East Colfax Avenue, Denver, Colorado 80206, USA).

14-15 January, **2nd Annual International Symposium on Sample Preparation and Isolation Using Bonded Silicas**, Philadelphia, USA (Mr Lane Yago, Symposium Coordinator, Analytical International Inc., 24201 Frampton Avenue, Harbor City, California 90710, USA).

21-22 January, **Symposium on Pediatric Pharmacology**, Ahmedabad, India (Professor S.M. Mansuri, Chairperson, Department of Pharmacology, B.J. Medical College, Amedabad 380016, Gujarat, India).

22 January, **Meeting on Radiological Protection Measurements and Their Traceability**, London (Prof. J.H. Martin, Programme Committee Secretary, Department of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee DD1 4HN, UK).

18-21 February, **International Conference on Health Hazards and Biological Effects of Welding Fumes and Gases**, Copenhagen, Denmark (Mr Richard M. Stern, WHO/EURO, 8 Scherfigsvej, DK-2100 Copenhagen Denmark).

22-24 February, **International Symposium on Conceptual Issues in Environmental Archaeology**, Oxford, UK (Conceptual Issues in Environmental Archaeology, Department of Geography, University of Strathclyde, Livingstone Tower, 26 Richmond Street, Glasgow G1 1XH, UK).